MYCOLOGIA

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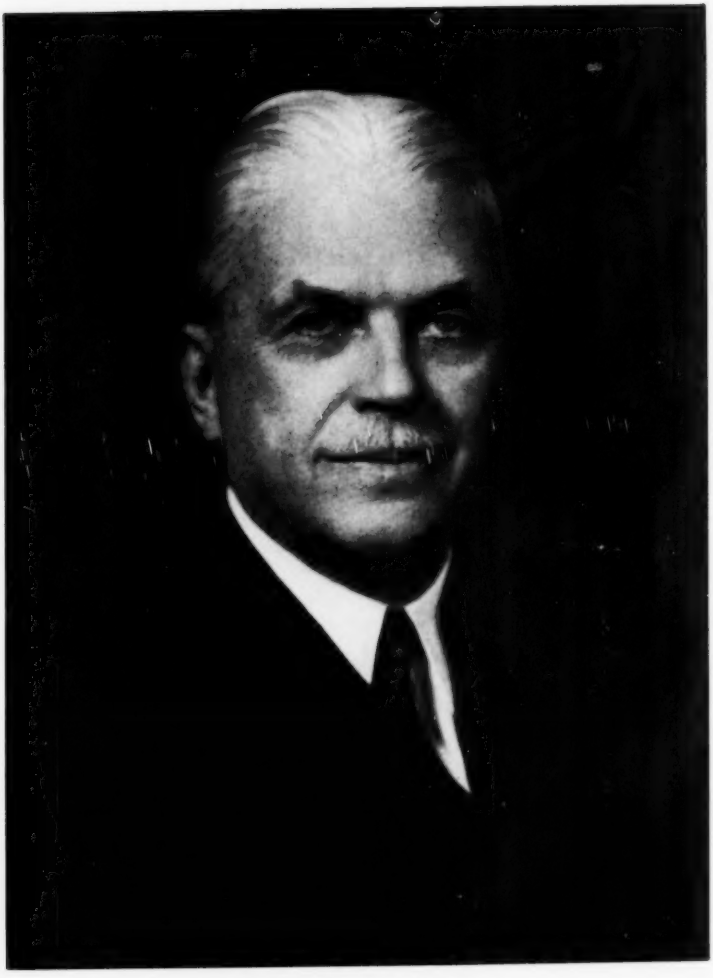

Bernard Ogilvie Dodge

Photograph by Bachrach

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THE FUNGI COME INTO THEIR OWN [1]

B. O. DODGE

(WITH PORTRAIT AND 1 FIGURE)

I appreciate deeply your invitation to speak to you this afternoon as you inaugurate a series of annual lectures. Perhaps what I have had in mind would have been more adequately expressed if the title given in the program had read: "Mycologists come into their own."

The time for preparation at my disposal was too short, however, to do justice to such a subject, so I shall leave it to others to give our mycologists their full due.

In his biographical sketch of the late Dr. Neil Stevens, Dr. Shear remarked that Stevens and he had published in the past several papers dealing with the mycological work and correspondence of Schweinitz, Curtis, Ravenel and Michener. Furthermore, during the many years of their close association they had accumulated a great collection of notes and letters in anticipation of publishing, some day, a "History of Mycology in America." Dr. Shear's botanical experiences in various capacities have extended back fifty years or more. He has a penchant for collecting all sorts of fungi. Supplemented by Dr. Stevens's youthful enthusiasm and refreshingly modern ideas concerning botanical contributors and mycological collections, we would have been assured that such a history would have been not only interesting, but, in itself, a valuable contribution to mycology.

[1] First annual lecture of the Mycological Society of America, Columbus, Ohio, September 12, 1950.

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No doubt they would have searched the highways and byways for records of amateur mycologists and collectors with their local lists of fungi and enviable local reputations as great scientists. They would have informed us all about the man Russell, who named a fine new bolete for his friend Frost, and about the man Frost, who named a fine new bolete for his friend Russell. *Boletus Frostii* and *Boletus Russellii* are mushrooms with *character*, even though they were described by amateurs.

You know there is a slight tendency among botanists to underestimate the value of local lists of fungi. One writer suggested that commenting on published reports, listing of species or describing new species, should be left to professionals and experts; in other words, "Shoemaker, stick to your last." Well, Charley Frost did stick to his last, cobbling and selling shoes for forty-nine years in the same shop in Brattleboro, Vermont. Yet he found time, for example, to collect and describe some twenty new species of *Boletus* in his list of New England fungi, published in 1874. One suspects that his "new species" would average up very well with the new species of *Boletus* still being described by our professional mycologists. Frost's contributions to the botany of New England were remarkable for one mostly self-educated, having few library facilities, books he had bought with funds made in his little shoe store. I am for encouraging the amateur mycologist.

About forty years ago I was preparing for my bachelor's "Thesis" a list, with notes, of the fleshy fungi I had collected in Wisconsin. In those days if you wanted to get your bachelor's degree in botany under Prof. R. A. Harper at the University, you had to submit a regular thesis typed on quality paper, four copies. How thrilled I was to come upon a list of Wisconsin fungi prepared by Dr. A. F. Bundy in 1877, and published in the United States Geological Survey! J. J. Davis, in his provisional list of parasitic fungi of the state, pointed out that since Bundy's collections had been destroyed by fire, his list of about thirty species of parasitic fungi had not been taken into account in subsequent enumerations. Paraphrasing the laundryman's retort to his customer without his ticket, we might say: "No specimens? list no good!" Other lists of fungi such as that of Underwood and Earle covering the Alabama fungi interest me even today.

Prof. Fitzpatrick, our former historian, in a semi-public lecture, "History of Mycology," given in 1929, said that according to Comes, the oldest known illustration of any fungus was that fresco painting of *Lactarius deliciosus* on the wall of Pompeii. Between 79 A.D. and the issue of Icones Farlowianae in 1929, thousands of plates illustrating various fungi in color have been issued. I feel that Farlow's illustrations of fleshy fungi have seldom, if ever, been surpassed. The *Farlow Herbarium, Farlow Library* and *Icones Farlowianae* are fitting memorials to his greatness.

Such volumes of illustrations are usually beyond the reach of the amateur. McIlvaine's "One Thousand American Fungi" is out of print. Perhaps the answer has been found in a stereoscope viewer for Kodachrome transparencies like Professor Smith's new machine. To try it out one day I stuck in Wheel No. 1 without noticing the name of the species shown in picture No. 1. At once I said, "Why, that is a perfect picture of *Barlea fulgens;* I found it in Schmeiling's woods near Algoma, Wisconsin, one day in the spring of 1904 or 1905." I am not certain as to what became of my specimens, but my notes on this curiously colored discomycete, published in the Transactions of the Wisconsin Academy, prove that *amateur* Dodge identified the species correctly. Smith has set a very good example of how to bring one phase of mycology to his students and to the public.

In *Life* magazine for April 10, you will find an article with three pages of pictures and description covering the life history of the so-called slime mold *Dictyostelium discoideum.* No author is named except that Roman Vishiac took the photographs. You know of the work of Harper, E. W. Olive, Raper, Bonner and others who studied these organisms from the standpoint of morphogenesis. While this article in *Life* magazine may not tell much that is new to science, it did give to a million or more people the chance to read for the first time about an organism that may some day be coaxed to reveal something about what it is that controls differentiation of organs in higher plants and animals.

CULTURING FUNGI

Fitzpatrick, in the 1929 lecture mentioned previously, seemed to imply that the study of fungi in culture in connection with taxo-

nomic monographs results in the splitting of species and the discarding of the older species concepts bases on single herbarium specimens. Now, on the basis of studies of pure cultures of species of *Sclerotinia,* Whetzel did split the old genus into several new genera, based, among other features, on the particular kind of conidial stage developed. The fault, if any, lay in stretching the value of the conidial stage in circumscribing a genus. Pure culture studies should result in reducing or "lumping" species.

A good example of this we find in the works of Thom and Raper. As a result of their sixty man-years of culture work on the Penicillia they reduced the number of named species from about 750 to 142, which they consider true Penicillia.

Kern, in a retiring address, points out that a very rapid advance in taxonomic mycology has resulted from the recent custom of growing the fungi in pure cultures. So today we find plant pathologists and mycologists everywhere culturing their fungi whenever possible. They certainly did show great wisdom and unselfishness in accumulating stocks of cultures which they have made available to others when called upon for personal work and work in connection with important emergency projects. For example, when there came calls in 1941 for cultures of strains of *Penicillium notatum* for studies on the production of the antibiotic penicillin, Thom and Raper were ready to supply not only cultures of thirty or forty strains of *Penicillium notatum* and a hundred or so strains of *P. chrysogenum,* but also 2000 cultures of strains of other species of *Penicillium.* This number was eventually increased to about 4000. Until 1941 you could not have found over a dozen *Penicillium* specialists even if you searched laboratories all over the world. But very soon there were hundreds, maybe thousands, of workers, if you include bacteriologists, chemists, pharmacologists and engineers, in addition to mycologists, who turned their attention to the Penicillia and other fungi.

Following the pressing demand for more information on species of this group, Raper and Thom published their Manual of the Penicillia which is a cyclopedia of information and a book that has few equals in taxonomic mycological literature.

The name of the bacteriologist who discovered penicillin is now familiar to millions of people; the one who gave us streptomycin

was widely known to microbiologists for his work on soil bacteria and soil fungi. He knew the streptomycetes long before their value as producers of antibiotics was even thought of.

As plant pathologist, active and retired, at The New York Botanical Garden, I have been encouraged to carry on research on life histories, cytology and genetics of the fungi without regard, in particular cases, to their pathogenicity to ornamental plants.

During the last decade, work on the physiology of fungi has greatly expanded at the Garden under the leadership of Professor Robbins. Here the old athlete's-foot and ring-worm fungi have been studied intensively, particularly as regards their tendency to mutate or "go pleomorphic." At the same time there has been carried on extensive work on antibiotics and bacterial viruses. Where for years only one member of the staff was culturing fungi, there are now about twenty, including chemists, bacteriologists and physiologists and culture technicians. This is but one example of similar work being pursued in many other institutions. To date, over 3000 strains or species of fungi and several hundred strains of Actinomycetes have been tested there for antibiotics. Now, where did the cultures of all these fungi come from? Who identified the fungi specifically? You will appreciate the value of the research that has been done by our pathologists and mycologists everywhere when you read this memorandum from Dr. Robbins answering my questions:

One hundred or more cultures have been received from the following individuals:

Ross W. Davidson, U.S.D.A., Beltsville, Md. (544);
H. S. Jackson, Univ. of Toronto (351);
Mildred K. Nobles, Central Experimental Farm, Ottawa (300);
José Lopes, Lisbon, Portugal (208);
W. Lawrence White, Army Service Forces, PQMD, Philadelphia (161);
H. H. Whetzel, Cornell University (160);
Dow V. Baxter, Univ. of Michigan (158);
Roger Heim, Paris (123);
Anna E. Jenkins, Bur. Plant Industry (110); and from
W. H. Wilkins, Botany Dept., Oxford University, Oxford, England (33).

The list of those who contributed fewer than one hundred cultures is too long to be read here. Nine institutions or central culture bureaus have also provided large numbers of cultures. These are located in countries as far apart as Dutch Guiana, China, Australia, India, Japan, New Zealand, Holland, Brazil and Argentina.

RESEARCHES ON DETERIORATION

We had to fight World War II, much of it in the tropics, to awaken the armed forces and industry to the great losses sustained due to damage done by the fungi. Clothing, tenting, blankets, cordage and leather goods rotted almost overnight. Insulation of electrical equipment soon broke down; cameras and other optical supplies often became useless; at least, their efficiency was greatly reduced. Research centers were established here and abroad to conduct deterioration tests of all kinds. Subdivisions of such stations called for highly trained mycologists in addition to personnel from other scientific groups. Dr. White, mycologist at Harvard, for just one example, was asked to head such a group. His papers published in MYCOLOGIA indicate how important and extensive was the work done on deterioration. So much is being done today along these lines that it looks as if a new science has been founded. *Here again the fungi have demanded attention and received it.*

MEDICAL MYCOLOGY

For many years research on the fungi that infect man was carried on mostly in Europe, particularly in France under Sabouraud, Langeron and others. Langeron's book, "Précis de Mycologie," is evidence that French students in medicine have been well instructed on the fungi. In America, early work on the fungi in medical colleges was mostly done by physicians who had had experience in the tropics where fungous diseases of man are more serious. It was back in 1918 that Colonel Schmitter, then stationed at the fort in Staten Island, came to Columbia University with a large trunk full of cultures which he had isolated from diseased service men as well as from natives of the Philippines and other tropical regions. He was very well informed as

regards dermatophytes, but he wanted to know about the fungi in general, their characteristics and classifications as plants, and where in such classes his pathogens belonged. Therefore he asked permission to join a class in the department of botany where he could get some of this information. Harvard, Tulane, Pennsylvania, Columbia, Duke and other medical colleges have introduced courses in mycology. Today it is not uncommon to find a mycologist holding a permanent position on the medical staff. The list of their published researches and books is a long one.

Although medical mycology is becoming more and more important, we hope that no one will want to start a new society, for example, American Medical Mycological Society. We old-time mycologists are just beginning to know something about the fungi that cause diseases in man, and medical men, like Colonel Schmitter, are learning a lot about those fungi that cause plant diseases, as well as those species that provide antibiotics or have other functions that should be known to physicians.

WHEAT RUST

The story of wheat rust is so well known to mycologists it does not need to be retold here. We owe much to Arthur, Kern, Jackson and their associates for extending de Bary's work on heteroecious rusts. We are all agreed that Arthur was our most noteworthy rust specialist.

Stakman and his associates, specializing on wheat rusts, used Arthur's methods, somewhat in reverse, to prove that *Puccinia graminis* exists in nature in a hundred or more physiological or biological races. This fact was more easily accounted for when Craigie made that most fundamental discovery that the pycniospores or spermatia function in fertilization.

As *Puccinia graminis* was found to be heterothallic the way was opened to effect hybridization resulting in new strains that differ in their pathogenicity. Genetic studies on rust parasites are becoming increasingly important every year.

Only one thing more now is necessary to make the story complete. I shall wait around until some biochemically-trained mycologist grows *Puccinia graminis* in culture free from its natural host plants.

Before leaving the rust parasites, I should like to mention two species which certainly would provide strategic material for study using modern techniques. One is *Gymnosporangium Ellisii*. This fungus infects the southern white cedar, *Chamaecyparis*. It invades the branches and large limbs, causing curious overgrowths, witches' brooms or stag-horns. As the mycelium develops in the cortex, the near-by parenchyma cells are stimulated to enlarge as each cell nucleus undergoes a few divisions. Space is provided as numbers of cells increase in size and push in and crush the normal cortex cells, thus simulating cancer metastases.

Another brightly colored fungous parasite that needs much further study is the orange-rust of blackberries and black raspberries. There are probably several distinct strains, all closely related, and all derived from some common ancestral form. Time does not permit mentioning the number of features that make these "orange-rusts" so well adapted for profitable research. For one example, it is very interesting to see that when a leaf, or any part of it, shows the rust in the spring, only that area showing rust pustules on the *lower* side develops stomata on the *upper* side. It is rather as if the parasite had signaled ahead to the host, that in order to live on from year to year, it would be wise to develop stomata on the *upper* side of infected leaves because the lower side is fated to be destroyed and so become non-functional. If this phenomenon is not a rarity, I shall be glad to learn of other examples.[2]

I should like now to mention other instances where the story of life histories of fungi has become more thoroughly understood because of the results obtained by the use of new methods or techniques.

ALLOMYCES ARBUSCULA

At our meeting held in New York last winter I heard a very interesting paper presented by Prof. Ralph Emerson on the life

[2] At the close of my lecture, Dr. V. M. Cutter, who had been present and heard these statements relating to the rust fungi, approached me with a chuckle and said, "What would you say, Dr. Dodge, if I told you we are already growing your old cedar apple *Gymnosporangium* rust in pure cultures in test tubes?" As you all know by now, Hotson and Cutter have since then published their paper announcing this discovery.

histories of certain water-molds from cytogenetic and cytotaxonomic standpoints. But what interests me most today is an earlier publication on: "The significance of meiosis in *Allomyces,*" by Emerson and Wilson.

They point out that Hans Kniep, in 1929, described a type of sexuality and life history in the water mold *Allomyces* previously unknown in the fungi. There appeared to be an actual alternation of generations, but chromosome counts were lacking to prove just where the haploid and diploid generations began and ended if there were such generations.

Using the squash technique and the aceto-orcein stain these authors proved that in *Allomyces arbuscula* meiosis occurs in the resting sporangium, which contains about a dozen diploid nuclei. A single zoospore gives rise to the gametophyte, the nuclei of which have seven chromosomes. Male and female gametangia are developed on the same thallus. A male and a female gamete fuse to initiate the diploid sporophyte with 14 chromosomes.[3] If you believe in the polyphyletic origin of the fungi you could no doubt find a red alga with a similar alternation of generations.

ALLOMYCES CYSTOGENUS

Allomyces cystogenus has quite a different life cycle. The only haploid cells in the cycle are those formed after reduction, at gametogenesis. There is only one mitotic nuclear division. The haploid number of chromosomes is seven.[3] The plant, like *Fucus,* is diploid.

As the authors point out, we have here in these two kinds of water molds excellent material for class use. Furthermore, as I shall call to your attention later, we have a somewhat comparable situation in the yeasts, *Saccharomyces cerevisiae* and *S. Chevalieri.* The former is very like *Allomyces arbuscula* and the latter is like *A. cystogenus,* in life cycle.

[3] Emerson, in Ann. Rev. Microbiol. 1950, discusses the limits of the haploid and diploid phases of *A. arbuscula* and *A. cystogenus* in the light of more recent studies. Referring to the latter species he says: "All parts of the life cycle are diploid with the exception of the spores from resistant sporangia, the gametangial cysts and the gametes." Wilson, Bull. Torrey Club **79**: 139–160. 1952, gives the haploid number of chromosomes of this species as 14.

NO HETEROTHALLISM IN PENICILLIUM PROVED

Some years ago Derx of France published the results of his culture work with *Penicillium luteum*. His data, as given, seemed to prove, as he claimed, that this species is heterothallic. Since heterothallism was known to be very common in all sorts of fungi, Derx's claim was accepted as a fact, until Emmons, who had studied in single-spore cultures the life histories of those species that produce perithecia, reported that *Penicillium luteum* is strictly *homothallic*. Raper and Thom, in all their culture work, have found no evidence that *P. luteum* or any other species of *Penicillium* is heterothallic. Those who have hoped to hybridize species of *Penicillium* to obtain new strains for antibiotic studies have cited Derx. If *P. luteum* is *heterothallic*, they say, *P. notatum* and *P. chrysogenum* may be strains of one mating type, and the other sex might be found by further search in nature. But *P. luteum* is not heterothallic. I am not saying that there may not be such a species discovered some day or that true hybrids between strictly homothallic races of these Penicillia can not be made.

HETEROCARYOSIS VERSUS HOMOCARYOSIS

It is very commonly stated that, where the hyphal branches of different strains or species of fungi meet in culture and *seem* to anastomose, nuclei migrate back and forth to develop a heterocaryotic mycelium. Whether or not the cells of the strains are uninucleate or multinucleate seems not to have been considered. While it is very easy to effect heterocaryosis in strains of *Neurospora tetrasperma*, I have never been able to do this when two *normal* strains of opposite sex of *N. sitophila* are grown together. This has been confirmed by Sansome, Beadle and others. Any claim that *N. crassa* exists in nature as self-sterile heterocaryons is not supported by experimental work. I question whether you can effect *true* heterocaryosis in *Penicillium notatum* if its mycelial cells are uninucleate. However, according to Pontecorvo, 1946, there may be many nuclei per cell in submerged hyphae.

THE DANGEARD-HARPER CONTROVERSY

Dangeard in the 1890's was the first to prove that asci, basidia, teliospores and smut spores originally contain two nuclei which fuse to form a diploid nucleus. This so impressed this brilliant young man that he stoutly maintained that the young ascus is an oogonium, the female nucleus of which is fertilized by the male nucleus. The primordia plainly seen in *Pyronema, Ascobolus* and *Penicillium* are merely trophogones, he said.

Harper, 1895–1905, strongly opposed Dangeard in a series of papers which attracted wide attention. The real sex-fusions and nuclear fusions occur at the origin of the ascocarp, the later fusion of nuclei in the ascus is merely compensatory. His theory was strongly supported by Helen Fraser (Dame Helen Gwynne-Vaughan) with evidence for a *first* reduction in the ascus at meiosis with a second reduction at brachymeiosis in the third division.

For many botanists, Claussen's beautifully illustrated paper, 1912, seemed to settle for all time this controversy. The nuclei come together in pairs in the oogonium, ascogenous hyphae develop, and finally the cells become binucleate, each nucleus being haploid. Yet we find that some botanists, even today, uphold the Harper theory.

Lindsay Olive, in a recent paper on *Lachnea melaloma,* an ascomycete studied by Gwynne-Vaughan, finds good proof, using the carmine technique, that there is only one nuclear fusion, and that one occurs in the ascus, whose primary nucleus is diploid and not tetraploid. Only one reduction was found in the ascus.

Finally, Hirsch, also using the squash-acetocarmine technique, has proved that the fusion nucleus in the ascus of *Pyronema* is diploid, with 24 chromosomes. A single reduction occurs, so that the haploid number is twelve.

Genetic studies on *Neurospora, Hypomyces, Glomerella* and other ascomycetes all prove without question that the theory of a double fertilization, followed by a double reduction, as a theory and as a fact, must be abandoned. *The classic Pyronema has at last been given full justice and a cleared reputation.*

In Sharp's "Fundamentals of Cytology," 1943, you will find a very clear and correct discussion of the life history of a hetero-

thallic ascomycete. Dr. Donald Rogers in his lecture before the Torrey Club last year pointed out that in many higher fungi there may be three very distinct phases of development—(1) a haplophase, often more or less vegetative; (2) a dicaryo-phase, the cells of which are binucleate, each nucleus being haploid; (3) a diploid phase which may be a very short one.

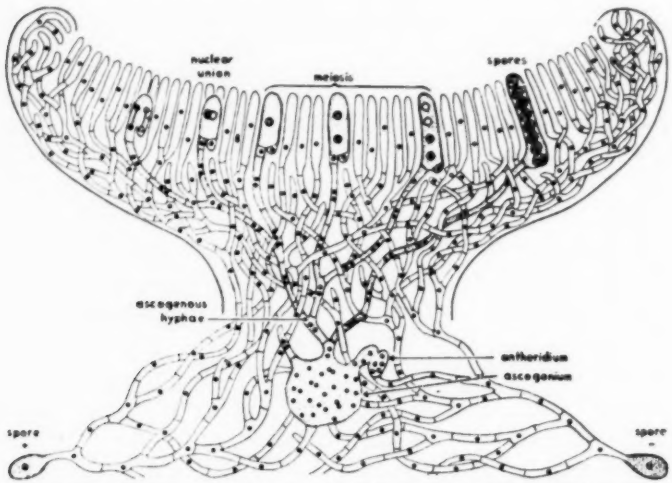

FIG. 121.—Diagram of nuclear history in the life cycle of a heterothallic ascomycete. Early and late stages of the cycle are shown, the early stages being in the lower portion of the diagram and later stages in the upper portion. Long before the apothecium and the spores are mature the sex organs have disappeared. The apothecium is composed of uninucleate hyphae and binucleate ascogenous hyphae. The arrangement of successive stages in the asci is arbitrary. In the ascus at the extreme left the last conjugate division is being completed. Natural proportions are not represented.

FIG. 1. Nuclear history of a heterothallic ascomycete. From Sharp, Fundamentals of cytology. McGraw-Hill Book Co.

Sharp, in his figure 121, which I am permitted to use here, shows all these phases distinctly (FIG. 1). All the essential features are brought out, namely, conjugate division, crosiers, asci and ascospores. Robbins and Weir in their new text-book have redrawn Sharp's figure with certain changes as shown in their text figure. Perhaps for the sake of simplicity, conjugate nuclear division, the formation of the young ascus with, first, two nuclei, then with one nucleus and nuclear divisions preceding spore formation, are all omitted. By labeling the binucleate ascogenous hyphae: "ascus-bearing hyphae (diploid $n + n$)," they have introduced a

serious element of confusion. The authors seem to be, in a sense, adopting the Harper-Gwynne-Vaughan terminology, because they were the ones who first referred to the nuclei in the ascogenous hyphae as *diploid*. A short explanation as to what Robbins and Weir mean by "diploid" and "$n + n$" could have been inserted in the text or in the legend for the figure.

PENICILLIUM GLAUCUM

For years most botanists have accepted Brefeld's work on *Penicillium glaucum* as illustrating various morphological features of a common or typical *Penicillium*. He figured a pair of intertwined perithecial initials, a sclerotioid perithecium, asci in chains, ascospores deeply grooved, and a typical conidial brush of spores, the penicillus.

Some years ago I found a very interesting species of *Penicillium*, the sclerotioid perithecium of which resembles, in section, that figured by Brefeld. However, the asci were not in chains but they arose as side-branches of hyphae developed in the lysigenous cavity of the ascocarp. The perithecium clearly had its origin, not from a pair of intertwined sex organs, but from non-ascogonial structures with characteristically branched elements. I described this new species under the name *P. brefeldianum*.

Raper and Thom have discussed Brefeld's work at length in their new Manual so that we may now conclude that Brefeld either worked with different species at different times, or with mixed or contaminated cultures. Brefeld used "clean" cultures which were not necessarily "pure" cultures. *"Penicillium glaucum" of Brefeld has received its just deserts. It has been rejected as a name for any particular species of Penicillium.*

The new Manual of the Penicillia now includes full descriptions of nineteen species that develop the ascocarpic stage, but we still lack a good illustration of the entire life history of some particular species. It should be diagrammatic so that it would be in demand by every one who writes a text-book of botany, mycology or antibiotics.

YEAST GENETICS

Fifteen years ago, 1935, the Danish geneticist Winge proved for the first time that our common yeast is diploid during its active useful vegetative stage. Any cell may become an ascus in which reduction occurs and four haploid ascospores are formed. Soon after these spores germinate two buds fuse after which their nuclei fuse. The now diploid yeast multiplies rapidly. This is a simple life history which writers of text-books of botany might, with justice to this little multibillion dollar fungus, include in their page on the yeasts.

Some years later, 1943, Lindegren proved that certain races of the species *Saccharomyces cerevisiae* are heterothallic, so that haploid races can be carried separately in cultures to be used as required in genetic studies. Haploid and diploid generations alternate.

Winge proved that *S. Chevalieri* is homothallic. Even so, it can be crossed with races of *S. cerevisiae* if one uses an ascospore of *S. Chevalieri* as one parent in the cross. Winge and Roberts, more recently, grew single ascospores of the latter species in cultures and used mass transfers of buds from such a culture as one parent in a cross with *S. cerevisiae*. When they analyzed the four races from each of several hybrid asci, they obtained what appeared to be a large percentage of tetrapolar asci, *AD, Ad, aD, ad,* as well as a small percentage of bipolar asci, either *AD, AD, ad, ad,* or *Ad, Ad, aD, aD*. This was proved to be a false picture because, when they inbred as well as back-crossed, they proved that in every case the supposedly haploid races *Ad* and *aD* were already diploid and so could not be crossed, that is, used directly as parents in a cross. *Their cells were already all diploid.*

This work of Winge and Roberts is also of great importance because it shows how you can hybridize a homothallic species of yeast with a heterothallic species, or even with another homothallic species if you use certain very necessary procedures. Since this is a fact, one can no longer doubt that one can hybridize species of homothallic higher fungi like *Pyronema* and certain strains of *Glomerella*.

GLOMERELLA GENETICS

Glomerella cingulata, which has been studied for many years by Edgerton and his associates, has been shown to be a most interesting fungus from the standpoint of its life history. Markert now confirms Edgerton and his colleagues as to the possibility of hybridizing two races even though each by itself produces a greater or less number of ascocarps. Markert suggests that since such races are actually homothallic the signs + and − should be abandoned. He also says that one should not expect to be able to effect heterocaryosis, because the hyphal cells are uninucleate. He has found that certain *Glomerella* races marked by certain biochemical factors can be crossed, and that, in some perithecia, the eight spores in an ascus are arranged four and four for these factors or in other asci all eight spores are alike, which shows that the four-four types of asci were hybrids, and the others were not hybrid asci.

No one had followed the early stages in perithecial development, but Wheeler, Olive, Ernest and Edgerton showed that in these central coils, some of whose cells contain only one nucleus, at least one cell has two nuclei and it is from such cells that the dicaryophase leading to growth of ascogenous hyphae arises. The fact is that no one knew at that time just where these two genotypically different nuclei came from.

McGahen, Wheeler and Edgerton presented a paper at our meeting in New York last winter, bearing the title: "The initiation and development of the perithecium in *Glomerella*." Unfortunately, I did not hear this paper and have not seen any published account of their findings. Very likely they have discovered just how these binucleate cells originate in a hybrid mating. *In any event Edgerton's work on Glomerella is rapidly coming into fruition.*

GENETICS OF NEUROSPORA

My own work on *Neurospora* has been confined to studies on heterothallism (obligate and facultative), inheritance of sex or mating type factors, mutations expressed as differences in morphological structures, truly dominant and recessive gene mutations,

heterocaryotic vigor, and lately to studies on possible ways one species may have arisen rather directly from another species.

Lindegren made an important contribution to *Neurospora* genetics when he proved that second-division segregation of factors in the ascus of *N. crassa* is due to a crossing-over. He also showed that certain factors are linked, and illustrated this for the first time by a six-point sex-chromosome map.

In the last decade a new and much more fruitful kind of genetics of the fungi has come into prominence with the work of Beadle, Tatum and their many associates and students. They refer to this as biochemical genetics. They have proved that normal growth in *Neurospora* requires some twenty amino-acid building blocks, about ten vitamins and other growth substances or essential metabolites. They say that *Neurospora* can synthesize all these except biotin, which Butler, Robbins and Dodge proved *Neurospora* could not make. Man must rely on other organisms to do it for him. Moreover, synthesis of these substances by *Neurospora* is carried on step by step, I understand; each step may be subject to being enhanced, retarded or blocked by genes which are heritable as units which can be located in linkage groups.

Houlahan, Beadle and Calhoun have assembled in tabular form data obtained by studies of *N. crassa* involving the dissection of 8549 asci of which 6905 were usable. The data cover 330 crosses involving 118 mutants. Sixty-nine genes representing at least 35 different loci have been placed in linkage groups, of which five are shown in Beadle's diagram. There are two more chromosomes yet to be mapped. Beadle's diagrams also show how they identify the particular metabolite which has been inactivated to produce a deficient mutant. They have in *Neurospora* tools for bioassay which may turn out to be second to none for such work. The use of similar biochemical units has been adopted recently in connection with studies of yeasts and other ascomycetes, and also with smuts and bacteria. *The results as reported are such as would have been unbelievable ten years ago.*

CORN SMUT

Working with corn smut, *Ustilago maydis*, David Perkins, a "biochemical geneticist," obtained 21 biochemical mutants and de-

termined the specific requirements of fifteen. He then made crosses and back-crosses, proving that his mutants were stable. No doubt, the use of biochemical mutants will greatly speed a better understanding of this parasite which Stakman and his associates have proved to be full of "biotypes" which show up as mutants and remain stable in culture. Perkins believes it will not be impossible to hybridize corn-smut *in vitro* after some one shows how it can be made to complete its life cycle in culture.

MISSING LINK

No one knows just where *Actinomyces* and *Streptomyces* should be classified among the fungi and bacteria.

Couch, as many of you know, has found a very interesting fungus which may represent the missing link that shows a connection between the bacteria and the lower fungi. *Actinoplanes philippinensis* develops a conidial stage much like that characteristic of *Actinomyces,* and, in addition, a sporangial stage that develops spores whose flagella stain with bacterial stains such as are said to distinguish bacterial flagella from those of the fungi.

No doubt Couch will have much to say in the near future on this important discovery.

SCHIZOMYCETES

If the last edition, 1949, of the Jordan-Burrows Bacteriology can devote a chapter of 70 pages to the pathogenic actinomycetes, molds, yeasts and other related organisms, we mycologists may be permitted to say a word about bacteria.

Farlow, in his retiring presidential address presented January 1, 1913, said, "The fact that forty years ago (1872) Sachs had never heard of bacteria, while today life has almost become a burden, one hears so much about them, is a striking instance of the rapidity of development of a subject having a practical as well as a theoretical value." Yet not even the brilliant Farlow could have dreamed that biochemical geneticists by 1950 not only would be hybridizing bacteria, but, in a sense, would be hybridizing viruses that kill bacteria.

Lederberg and Tatum, 1946/7, using techniques they had employed so successfully in previous years in genetic studies in

Neurospora, actually hybridized strains of *Escherichia coli.* By growing together strains marked by certain biochemical deficiencies they recovered recombinant races as well as the parental races in such ratios as to indicate Mendelian segregations. Furthermore, the fifteen or more factors governing the synthesis of the metabolites employed appeared to be linked together in a single linkage group corresponding to the single chromosome now proved to be present in this species.

Lederberg in his 1947 paper shows how he located two or three genes that appeared to be linked, then three or four more showing linkage, and finally proved that they are all in one linkage group. Thus he showed for the first time an eight-point genetic chromosome map for bacteria.

Erwin F. Smith would have felt fully repaid for his years of work if he could have looked forward to this day and have seen crown gall still being studied in the hope of finding a clue to solution of the cancer problem. Brown, White and others are now growing crown gall in tissue cultures free from the bacteria. This may prove to be a big step in advance.

So we can say with assurance that the *Schizomycetes or fission fungi are not the degenerates that they have heretofore been reputed to be.*

BACTERIOPHAGES

Even as an outsider, I have been interested in the work of Luria, Delbrück, Hershey, Rotman and others on bacterial viruses. Dr. G. W. Beadle, in Science in Progress, 1949, has commented on their papers, especially on one by Hershey and Rotman, reporting their genetic experiments with phage recombination in *Escherichia coli.* At the left, in Beadle's diagram based on their data, is shown a bacterial cell in contact with which are two mutant particles differing in their ability to control the amount of lysis and host range. Within the cell the virus particles multiply, the cell is lysed, and a hundred or more particles are dispersed, as shown at the right. The authors by highly refined techniques were able to recover not only both parental types but also two recombinant types in the percentages indicated.

Beadle comments further as follows: "It appears that bacterial viruses are multigenic systems that lead some sort of sex life, recombining their component hereditary units in a manner not unlike that of higher cellular forms. There is evidence that phage genes are organized in linkage groups of some kind."

I suppose the next thing we shall be hearing will be about some virus disease of *Neurospora,* and about its mycophages that can be hybridized to develop new and more deadly strains which the bakers can use instead of chemicals to keep their bread from molding.

So I shall wait around until this is done, and then I can say: *"The old red bread-mold has at last come into its own."*

THE NEW YORK BOTANICAL GARDEN,
NEW YORK 58, N. Y.

ANTIBIOTIC SUBSTANCES PRODUCED BY SPECIES OF CEPHALOSPORIUM, WITH A DESCRIPTION OF A NEW SPECIES

JOHN M. ROBERTS

The mold strain M.D.H. *3590A* which forms the antibiotic synnematin was originally assigned to the form-genus *Tilachlidium* of the *Stilbellaceae*, and the name of the antibiotic was based on the characteristic synnemata of the mold (**5**). It was recognized that the formation of synnemata was a minor difference upon which to separate the form-genus *Tilachlidium* from the form-genus *Cephalosporium*. Dodge (**2**) and Ehrlich (**3**) both felt the difference was insufficient to be considered the basis for separation of the two genera, since a number of recognized *Cephalosporium* spp. also form synnemata on some media. Because of the questionable identity of M.D.H. *3590A* and the close relationship between the two genera, species of both *Cephalosporium* and *Tilachlidium* were examined in the search for other synnematin-producers.

MATERIALS AND METHODS

A comparison was made of three cultures isolated at the Michigan Department of Health (M.D.H. *602*, M.D.H. *2653*, and M.D.H. *3590A*); four species of *Tilachlidium* and thirty-four of *Cephalosporium* from the Centraalbureau voor Schimmelcultures; two species of *Cephalosporium* from the American Type Culture Collection; and one identified and five unidentified cultures of *Cephalosporium* and one unidentified species of *Tilachlidium* from the Philadelphia Quartermaster Depot. The sources of the individual cultures are noted in TABLE III.

Preliminary comparisons of the antibacterial activities of the molds were made by the cross-streak plate method. The molds were grown for six days at 24° C. on plates of F.D.A. agar,[1] after

[1] "F.D.A. agar" denotes penicillin assay agar according to the F.D.A. formula (**4**). "F.D.A. broth" refers to the nutrient broth portion of this agar.

which the plates were streaked with liquid suspensions of bacteria and incubated at 37° C. for 48 hours. The test organisms were *Micrococcus pyogenes* var. *aureus* (strain *209P*), *Salmonella typhimurium* (Dr. P. R. Edwards, strain 9), *Escherichia coli 621*, and *Mycobacterium 607*. The results were recorded as the distances in millimeters from the edge of the mold growth to where there was evidence of growth of the test bacteria.

Broth cultures of the molds were shaken at 24° C. in 25 ml. volumes in 250 ml. Erlenmeyer flasks at 240 r.p.m. on a rotary shaker with a 1½ inch stroke. Four liquid media were compared:

1. F.D.A. broth (the broth portion of penicillin assay agar)
2. Casamino Acids—Lactose broth (**5**)
3. Soy Meal broth

Soy bean meal	20.0 g.
Lactose	2.0
$FeSO_4 \cdot 7H_2O$	0.0025
$MgSO_4 \cdot 7H_2O$	0.125
Tap water	1000.0 ml

pH 6.8 before sterilization

4. Soy-Corn broth

Soy bean meal	20.0 g.
Corn meal	10.0
$FeSO_4 \cdot 7H_2O$	0.0025
$MgSO_4 \cdot 7H_2O$	0.125
Tap water	1000.0 ml

pH 6.8 before sterilization

Samples from the shake flasks were tested for antibacterial activity against *M. pyogenes*, *S. typhimurium*, and *E. coli* by a modification of the one-dimensional agar diffusion assays described by Masuyama (**6**) and by Mitchison and Spicer (**7**). The test agar was the F.D.A. formula from which glucose had been omitted in order to minimize the formation of gas bubbles. Two hundredths percent of a 24-hour F.D.A. broth culture of the test organism was added to the melted agar; and the agar was dispensed in 2.0 ml. volumes in 12 × 70 mm. test tubes. The agar was allowed to harden 15 minutes or more at 5° C., after which 0.4–0.6 ml. of the sample was added. To prevent the formation of secondary areas of growth in the clear inhibition zones of the agar, the tubes were held at 5° C. for 2 hours after adding the samples. The results were read after 18 to 24 hours at 37° C. and were recorded as

millimeter inhibition; i.e., the distance from the agar-liquid interface to the point of growth of the test organism in the base of the tube.

Cultures of the molds for comparison of pigment-formation and morphology were grown in duplicate on F.D.A. and on Czapek-Dox agar.

EXPERIMENTAL RESULTS

TABLE I presents the results of the cross-streak plates. Only those strains which showed antibiotic properties are listed. Since the varying degrees of inhibition of *M. pyogenes* by these strains could result from differences in concentration of similar products,

TABLE I

INHIBITION OF BACTERIAL GROWTH BY *Cephalosporium* STRAINS ON CROSS-STREAK PLATES

Species or strain number	Millimeters inhibition of test bacteria			
	M. pyogenes 209P	*S. typhimurium*	*E. coli*	*Myco. 607*
C. charticola Lindau	14, p21*	14	—**	—
C. lamellaecola Smith	p13	—	—	—
C. lanoso-niveum v. Beyma	1, p6	11, p13	—	—
C. sp. QM *107a*	7, p11	—	—	16, p21
C. sp. QM *127e*	2, p14	—	—	16
M.D.H. *602*	7	—	—	14, p20
M.D.H. *2653*	14	—	—	21
M.D.H. *3590A*	18, p22	18, p23	p10	16, p22

* p indicates partial inhibition.
** —indicates no inhibition.

prime consideration was given to the anti-*typhimurium* and anti-*607* activities.

Preliminary tests of broth cultures of the M.D.H. strains showed that the inhibitory principle against *Mycobacterium 607* was not formed in the same liquid media as was that against *M. pyogenes, S. typhimurium,* and *E. coli.* Because of this the anti-*607* activity was not included in the concept of synnematin. Further comparison of the 50 strains in shaken broth cultures was confined to those liquid media in which M.D.H. *3590A* formed synnematin.

Only the three M.D.H. strains, *C. charticola,* QM *107a,* and QM *127e,* produced culture fluids which inhibited *S. typhimurium.*

TABLE II

ANTIBACTERIAL ACTIVITY OF *Cephalosporium* SHAKE CULTURE FLUIDS

Species or strain	Medium											
	F.D.A.			Casamino Acids			Soy			Soy-Corn		
	M. pyogenes	*S. typhi-murium*	*E. coli*	*M. pyogenes*	*S. typhi-murium*	*E. coli*	*M. pyogenes*	*S. typhi-murium*	*E. coli*	*M. pyogenes*	*S. typhi-murium*	*E. coli*
C. charticola	−	−	−	−	+	−	++	+++	−	+	++	−
QM *107a*	−	−	−	+	++	−	+++	+++	−	++	+++	−
QM *127e*	−	−	−	−	−	−	+	−	−	+	++	−
M.D.H. *602*										+	++	−
M.D.H. *2653*							++	++	−	+++	+++	+
M.D.H. *3590A*	−	++	−	+	++	−	+	++	−	+++	+++	++

− indicates no activity.
+ indicates test bacterium inhibited 1–3.75 mm. (up to 2 dilution units/ml.).
++ indicates test bacterium inhibited 4–6.75 mm. (3 to 8 dilution units/ml.).
+++ indicates test bacterium inhibited 7 or more (10 or more dilution units/ml.).

All six of these were included among those cultures which showed activity on cross-streak plates. *C. lanoso-niveum* was inactive in broth although this species had inhibited *M. pyogenes* and *S. typhimurium* on plates. Strains M.D.H. *602*, M.D.H. *2653*, QM *107a*, and QM *127e* which had not inhibited *S. typhimurium* on plates did form an anti-*typhimurium* principle in liquid culture. The similarity of the broth activity patterns indicated that all six of the strains formed synnematin or a synnematin-like substance in one or more of the media used. It has already been noted by Gottshall *et al.* (**5**) that *C. charticola* does form synnematin. The possibility that synnematin, as originally described or as investigated here, may be a complex rather than a single substance has not been disproved.

IDENTITY OF M.D.H. 3590A

The supposition that strain M.D.H. *3590A* might be a member of the genus *Cephalosporium* instead of a *Tilachlidium* species was confirmed on comparison of this strain with identified mold cultures obtained from authoritative collections. Crandall (**1**) has stated that Corda's original description of *Cephalosporium acremonium* is inclusive enough to contain "almost any *Cephalosporium*." In addition to being in agreement with Crandall in this, the author feels that conidial sizes and shapes in *Cephalosporium* are so variable that they alone are insufficient to delineate the species.

Although this paper is not an attempt to emend or to monograph the genus, TABLE III is submitted as an aid in rapid comparison of species of *Cephalosporium*, and of *Tilachlidium* for those who would maintain this genus in its individual status, on the basis of colonial structure and pigmentation. For additional information, the antibacterial activity on agar and in shaken cultures is indicated. Colors were selected on the basis of Ridgway's Color Standards, and are given only for those species which are not all white.

Strain M.D.H. *3590A* had shown some similarities with descriptions of other species of *Cephalosporium*, but direct comparison of cultures brought out differences between this strain and the others.

TABLE III

COLONIAL AND ANTIBIOTIC CHARACTERISTICS OF *Cephalosporium*

Name	Colonial Characteristics						Antibiotic Activity	
	F.D.A. Agar			Czapek-Dox Agar			Agar	Broth
	Pigment	Synnemata	Habit	Pigment	Synnemata	Habit		
Cephalosporium: acremonium Cda. (Gruner) *	salmon color	+	appressed	pale salmon color	+	appressed	—	—
acremonium Cda. (Janke) *	flesh color	—	appressed, wet	shell pink	—	appressed, wet	—	—
acremonium Cda. (Koehler) *	salmon-buff	—	appressed, wet with white bloom	pale salmon	+	interwoven ropes	—	—
album Preuss *	pale salmon	small	appressed, wrinkled	seashell pink	+	appressed, powdery	—	—
asteroides Grutzii Ben. *		+	appressed, wet	pale flesh	+ large	appressed, wet	—	—
ballagii Oomen *		—	appressed, wet with white bloom	capucine buff areas	+	raised, damp	—	—
carpogenum Ruehle *		—	interwoven ropes		—	interwoven ropes	—	—
charticola Lindau *		—	thickly interwoven	neutral-red reverse	—	thickly interwoven	+	+

TABLE III—*Continued*

Name	Colonial Characteristics						Antibiotic Activity	
	F.D.A. Agar			Czapek-Dox Agar			Agar	Broth
	Pigment	Synnemata	Habit	Pigment	Synnemata	Habit		
Cephalosporium: ciferri Verona *		—	appressed with interwoven bloom	pale pinkish buff	—	interwoven	—	—
coccidiocolum Gueguen *		—	powdery	massicot yellow	+	interwoven	—	—
coccorum (Petch) Westerdijk *		—	furry		—	furry	—	—
curtipes Sacc. *		—	appressed with interwoven ropes over surface		—	interwoven ropes	—	—
deformans Crandall *	buff areas	—	long interwoven ropes	pale cinnamon pink	—	long interwoven ropes	—	—
furcatum M. et F. Moreau *		+ small	appressed, wet		+	granular	—	—
gramineum Nisik & Skata *		+	appressed, wet	light buff	+	appressed, wet	—	—
lamellaecola Smith *		—	furry	hazel reverse	—	furry	+	—

TABLE III—*Continued*

Name	Colonial Characteristics						Antibiotic Activity	
	F.D.A. Agar			Czapek-Dox Agar			Agar	Broth
	Pigment	Synnemata	Habit	Pigment	Synnemata	Habit		
Cephalosporium: *lanoso-niveum* v.Beyma *		—	furry	neutral red reverse	—	furry	+	—
lecanii (Zimm.) Viegas *		—	furry		—	furry	—	—
lefroyi Horne *		+ very small	powdery	warm buff reverse	+ very small	interwoven ropes	—	—
longisporum Petch *		—	interwoven ropes		—	interwoven ropes	—	—
malorum Kidd *	pinkish	—	appressed with granular surface		+ very small	wet	—	—
niveo-lanosum Ben. *		—	furry		+ fine, branched	furry	—	—
pseudofermentum Cif. *		+	appressed, wet	pale pinkish buff areas	+	interwoven ropes	—	—
recifei de Leao & Lobo *		—	appressed, granular, wet	greenish area	—	appressed, wet	—	—

TABLE III—*Continued*

Name	Colonial Characteristics						Antibiotic Activity	
	F.D.A. Agar			Czapek-Dox Agar			Agar	Broth
	Pigment	Synnemata	Habit	Pigment	Synnemata	Habit		
Cephalosporium: rubescens Schimon *		+ large	appressed		—	appressed, wet	—	—
rubrobrunneum-cerebriforme-hartmanii Ben. *	buff-pink	+ small	appressed, wet	pale flesh color	—	appressed, powdery	—	—
sclerotigenum M. & F. Moreau (CBS) *		—	granular		—	granular	—	—
sclerotigenum M. & F. Moreau (Moreau) *		—	granular		—	appressed with interwoven surface	—	—
sclerotigenum M. & F. Moreau (Valenta) *		—	granular	pinkish areas	—	granular	—	—
serrae (Maffei) v. Beyma (Verona) *		—	powdery		—	interwoven and granular	—	—
stühmeri Schmidt et v. Beyma *		—	interwoven ropes	pale cinnamon pink areas	+ very small	interwoven ropes	—	—

TABLE III—*Continued*

Name		Colonial Characteristics						Antibiotic Activity	
		F.D.A. Agar			Czapek-Dox Agar			Agar	Broth
		Pigment	Synnemata	Habit	Pigment	Synnemata	Habit		
Cephalosporium: subverticillatum Schulz & Sacc.	*		—	appressed, granular with interwoven strands		—	furry, with interwoven strands	—	—
tabacinum v. Beyma	*		—	appressed, wet		—	granular with interwoven strands	—	—
spinosum Negroni	*		+	appressed, wet		+ short & stout	wet	—	—
kiliense ATCC *4301* Syn. = *C. asteroides Grutzii*	**		+	appressed, wet		+	appressed, tufted		
diospyri Crandall ATCC *9066*	**		—	appressed, powdery		—	appressed, wet		
acremonium QM *611*	***	vinaceous-cinnamon	—	appressed, wet, much subsurface		—	mostly subsurface	—	—

TABLE III—*Continued*

Name	Colonial Characteristics						Antibiotic Activity	
	F.D.A. Agar			Czapek-Dox Agar			Agar	Broth
	Pigment	Synnemata	Habit	Pigment	Synnemata	Habit		
Cephalosporium: *sp.* QM *107*a ***		+		salmon color	+		+	+
sp. QM *127e* ***	tan	+ small	wrinkled	snuff brown (also in agar)	—	appressed, with interwoven strands	+	+
sp. QM *124 h* ***		—	appressed, wet except for conidiophores		—	interwoven strands	—	—
sp. QM *597* ***		—	interwoven strands	greenish	—	interwoven strands	—	—
sp. QM *598* ***	buff pink	+ small	appressed, wet	buff pink	+ very small	appressed		
sp. MDH *602*	seashell pink	+	smooth, granular to cottony	pale salmon color	+	smooth, dry, cottony	+	+
sp. MDH 2653	seashell pink	+	smooth, granular to cottony	pale salmon color	+	smooth, dry, cottony	+	+

TABLE III—*Continued*

Name	Colonial Characteristics						Antibiotic Activity	
	F.D.A. Agar			Czapek-Dox Agar			Agar	Broth
	Pigment	Synnemata	Habit	Pigment	Synnemata	Habit		
Cephalosporium: sp. MDH 3590A	buff-pink to salmon color	+	appressed, sometime zonate	salmon color to pale salmon color, flesh ochre reverse	+	smooth, dry, sometime zonate	+	+
Tilachlidium: butyri v. Beyma *		+	appressed, wet, granular surface	olive (also in agar)	—	interwoven strands, granular	—	—
tomentosum (Schrod.) Lindau (CBS) *		—	appressed, wet		—	furry	—	—
nigrescens El. et Em. Marchal *		+	appressed, wet	walnut brown (also in agar)	—	appressed, wet	—	—
tomentosum (Schrod.) Lindau (Truter) *		+	appressed, wet	pale vinaceous pink areas	—	appressed, powdery	—	—
sp. QM *619* ***	(failed to grow)			(failed to grow)				

* Culture obtained from Centraalbureau voor Schimmelcultures, Baarn.
** Culture obtained from American Type Culture Collection, Washington.
*** Culture obtained from U. S. Army Quartermaster Depot, Philadelphia.

On the basis of colonial character and pigment M.D.H. *3590.A* most closely resembled *C. rubrobrunneum-cerebriforme-hartmanii* Benedek. However, the synnemata, conidiophores, and conidia of M.D.H. *3590.A* were larger than those of *C. rubrobrunneum* on both F.D.A. and Czapek-Dox agar. Pigmentation was more pronounced in the former on both media. The cytoplasm of M.D.H. *3590.A* conidia was evenly granular in contrast to large clear areas partially surrounded by crescents of oily material in conidia of *C. rubrobrunneum*. The combined macroscopic and microscopic differences between these two molds were evidence that M.D.H. *3590.A* constitutes a new species of *Cephalosporium*.

Cephalosporium salmosynnematum n.sp.

Coloniae in agaro Czapeki-Doxio salmoneae ad pallide salmoneae, albopubescentes; synnemata disseminata, interdum anulos concentricos formantia, e basi 55–90 μ lata apicem versus gradatim attenuata, 420–900 μ alta, ex hyphis longitudinalibus interextis composita; conidiophora per totam longitudinem synnematis nascentia, 14–38 μ longa, 0.75–1.5 μ diametro ad basim; conidia ellipsoidea vel ovoidea, 3.4–6.4 $\mu \times$ 2.3–3.4 μ hyalina, in capitulis mox dispersis aggregato.

Ex contaminatione in laboratorio, Lansing, Michigan.

Specimen typicum: M.D.H. *3590.A* in Michigan Department of Health and Northern Regional Laboratories.

On beef extract-peptone-yeast agar: Colony buff pink to salmon color, sometimes with concentric rings of whitish pubescence; synnemata gregarious near center of colony, mostly erect, 900–1200 μ long, 50–100 μ in diameter at base tapering to a single hypha at tip, composed of parallel and interwoven hyphae which are 1.5–2.7 μ in diameter; conidiophores arising along entire length of synnema, non- or 1-septate in addition to basal cross-wall, 45–100 μ long, unbranched, 1.8–2.3 μ in diameter at base, tapering to 1.0–1.5 μ at tip; conidia ellipsoid or ovoid, 4.6–7.7 $\mu \times$ 2.7–3.8 μ hyaline, borne in heads which are easily dispersed; conidia and hyphae vacuolate and containing few orange-tinged granules.

On Czapek-Dox agar: Colony salmon color to pale salmon color, with whitish pubescence; synnemata scattered, occasionally forming concentric rings, mostly erect, 420–900 μ long, 55–90 μ in diameter at base tapering to a single hypha at tip, composed of parallel and interwoven hyphae which are 1.5–3.1 μ in diameter; conidiophores arising along entire length of synnema, septate only at base, 14.0–38.0 μ long, unbranched, 0.75–1.5 μ in diameter at base, tapering to 0.5–0.75 μ at tip; conidia ellipsoid or ovoid, 3.4–

6.4 $\mu \times$ 2.3–3.4 μ, hyaline, borne in heads which are easily dispersed; cell contents of conidia evenly granular, of hyphae mixture of small oil globules and orange-tinged granules; hyphae of synnemata especially filled with inclusions.

Originally isolated as a laboratory contaminant at the Michigan Department of Health, Lansing, Michigan.

Type: Strain M.D.H. *3590A* at Lansing, Michigan, and as sent to the Northern Regional Research Laboratories, Peoria, Illinois.

The most striking characteristics of *Cephalosporium salmosynnematum* are the rich salmon pigment of the colony and the clustered erect synnemata. On agar plates and on the surface of stationary broth cultures the circular colonies have clearly-defined margins, and the symmetry of the circular edge is seldom broken by irregularities. The surface is usually flat except for the synnemata, although old cultures occasionally form radiating ridges of growth. In very old cultures some autolysis of the mycelium occurs with the formation of a pigmented gelatinous mass which underlies the intact filaments. Not infrequently individual hyphae and small strands of synnemata are found to coil back on themselves to form circles of filaments. No anastomosing nor sexual bodies have been distinguished in the vicinity of these circles in M.D.H. *3590A*.

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SUMMARY

1. Antibacterial activity against *Micrococcus pyogenes* var. *aureus, Salmonella typhimurium, Escherichia coli,* and/or *Mycobacterium 607* was demonstrated by eight strains of *Cephalosporium* on agar plates.

2. Six of these eight strains formed synnematin or a synnematin-like substance in shaken broth cultures.

3. Forty-two other strains of *Cephalosporium* and *Tilachlidium* showed no antibacterial activity.

4. The name *Cephalosporium salmosynnematum* is proposed for strain M.D.H. *3590.A.*

DIVISION OF LABORATORIES,
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PRODUCTION OF RIBOFLAVIN BY EREMOTHECIUM ASHBYI GROWN IN A SYNTHETIC MEDIUM [1]

KATHERINE E. YAW [2]

The yeast-like ascomycete, *Eremothecium ashbyi,* which is parasitic upon cotton bolls, was described for the first time by Guillermond (**3**) in 1935. A great deal of the recent interest in this fungus has been due to the large amount of fluorescent yellow pigment it produces which was identified by Raffy (**8, 9**) as riboflavin.

The growth factor requirements of this fungus have been investigated by various workers. Schopfer (**11**) found biotin to be an essential growth factor and inositol and thiamin accessory or complementary factors. In addition to these, there was an unknown factor present in most peptones and other natural substances which was necessary for the growth of this microorganism. Moore and de Brecze (**7**) grew *E. ashbyi* in a synthetic Czapek sucrose broth and did not mention the addition of any growth factors. Schopfer and Guilloud (**12**) reported that the addition of leucine or arginine alone or in combination allowed the growth of *E. ashbyi* in a synthetic medium to which biotin, thiamin and inositol had been added. Dulaney and Grutter (**2**) reported inositol to be the only essential growth factor for this fungus if the nitrogen source was L-arginine, DL-glutamic acid or L-proline in amounts higher than 0.1%. They reported also that the addition of various components of the vitamin B complex did not increase the production of riboflavin by *E. ashbyi.*

[1] From a portion of a dissertation in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Yale University. It is a pleasure to acknowledge the help and encouragement offered by Drs. P. R. Burkholder, E. L. Tatum, D. M. Bonner, V. M. Cutter, Jr., and other members of the laboratory where this work was done.

[2] United States Public Health Service Junior Research Fellow.

This paper presents different results on the growth factor requirements of *E. ashbyi*. The effect on riboflavin production of the addition of various compounds to the synthetic medium is also reported.

MATERIALS AND METHODS

The culture used in this work was obtained from Dr. W. H. Schopfer. Three single spore isolates were made and since they appeared identical in colonial form, morphology, and yield of riboflavin, one of the isolates, *2*, was chosen arbitrarily for further experimental use. Other strains of *E. ashbyi* were obtained from the New York Botanical Garden, Northern Regional Research Laboratory, Lederle Laboratories, and Commercial Solvents, Inc., laboratory. These strains were tested only for their growth factor requirements.

The medium used throughout this work for the culture of *E. ashbyi* was the one employed by Schopfer (**11**) in his work on the essential growth factors for this fungus. It contained the following:

Glucose, C. P.	10.0 g.	Biotin	2.5 μg.
$MgSO_4 \cdot 7H_2O$	0.5 g.	B_1	400.0 μg.
KH_2PO_4	1.5 g.	Inositol	40.0 mg.
Asparagine (twice recrystallized)	1.0 g.	Distilled water	1000.0 ml.

The inositol listed above was found by Schopfer to be an accessory growth factor but in this work and in that of Dulaney and Grutter (**2**) it was found to be essential for growth.

At pH values higher than the original medium the KH_2PO_4 was replaced by M/150 KH_2PO_4 and M/150 Na_2HPO_4. The pH adjustments were made with the salts at M/15 according to the standard phosphate buffer solutions (**4**) and then diluted 1–10.

The constituents of the various stock dilutions which are given below were used in this work either in establishing the growth factor requirements of the fungus or in determining factors influencing the production of riboflavin. They were added in varying amounts to the minimal medium given above.

A. Stock solutions of B vitamins in amounts added per 100 ml. of distilled water:

Thiamin·HCl	10 mg.	Choline·HCl	100 mg.
Riboflavin	5 mg.	Niacin	10 mg.
Pyridoxin·HCl	5 mg.	Folic acid	1 mg.
Ca-pantothenate	50 mg.	Biotin	50 μg.
p-Aminobenzoic acid	5 mg.	Inositol	100 mg.

B. Essential (**10**) amino acid mixture containing 1 mg. of each of the following per 100 ml. of distilled water:

L-valine	DL-isoleucine
DL-methionine	DL-threonine
L-histidine·HCl	L-tryptophane
L-arginine·HCl	DL-phenylalanine
L-lysine·HCl	DL-leucine

C. Non-essential (**10**) amino acid mixture containing 1 mg. of each of the following per 100 ml. of distilled water:

D-glutamic acid	L-cystine
L-aspartic acid	DL-alanine
DL-serine	L-tyrosine
L-proline	glycine

D. Hydrolysed yeast nucleic acid (**6**) which contained 10 per cent nucleotides or 100 mg. of nucleotides per ml.

The cultures were grown either in 5 ml. of medium per 18 × 140 mm. test tubes or in 20 ml. of medium per 125 ml. Erlenmeyer flasks. For inoculum the fungus was grown in 3% glucose plus 2% yeast extract broth, centrifuged after 5 or 7 days growth, the cells taken up in sterile distilled water in twice the original amount of liquid and then one loopful of this suspension was used to inoculate each tube or flask. The tubes, held in wire racks, and the flasks were put on the mechanical shaker in the 30° C. room and allowed to grow for 7 days. The shaker moved back and forth at the rate of 80 complete cycles per minute.

All assays in the work reported were run colorimetrically, using a Klett-Sumerson colorimeter. The colorimetric method was checked with the Snell and Strong (**13**) microbiological assay, using *Lactobacillus casei* ε, and the values obtained were found not to vary more than ± 5%.

EXPERIMENTAL

Growth factor requirements. Various combinations of growth factors such as a mixture of B vitamins, essential and non-essential amino acids and hydrolyzed yeast nucleic acid were added to the synthetic medium given above in amounts found to be effective for other microorganisms. None of these additions was able to support the growth of the fungus singly or in combination. Many other substances including oleic acid, indole-3-acetic acid, glutathione, xanthine, hypoxanthine, and strepogenin (**14**) were added and found to be ineffective.

TABLE I

EFFECT OF VARIOUS COMPOUNDS IN REPLACING METHIONINE IN *E. ashbyi*
0.1 mg. of compound added per 5 ml. of medium pH 5.8

Compound added	Amount of growth*
DL-Methionine	++++
Homocystine**	0
Homocysteine**	+++
L(−)-Cystine**	0
L-Cysteine**	+++
Sodium thioglycollate**	+++

* Macroscopic examination: ++++, excellent; +++, good; ++, fair; +, poor; 0, no growth. These designations will be used throughout the paper.
** 0.1 mg. of choline added also.

While testing various other synthetic media than that one already given it was found that *Neurospora* minimal medium (**1**) plus 0.1% N–Z Case would support the growth of *E. ashbyi.* The pH of this medium is 5.7 whereas the pH of the Schopfer minimal medium is 5.0. It was found that the addition of the essential amino acid mixture would support the growth of the fungus in Schopfer synthetic medium if the pH of that medium was raised to 5.8. The ten components of the mixture were added in 0.1 mg. amounts to determine which one or ones were essential. It was found that the addition of methionine alone supported growth. After the work of Lampen *et al.* (**5**) on the biosynthesis of methionine by *Escherichia coli,* it was decided to test the compounds used by those workers for their ability to replace methionine in a fungus which required that amino acid. Possible explanations for the results shown in TABLE I are given later in the discussion.

After determining the substance which permitted growth at pH 5.8 it was necessary to find what compounds would support growth at lower and higher pH values. These requirements are presented in TABLE II. From the results it can be seen that there is a pH range where no additional growth factors are required, whereas at other levels of pH many are needed if the fungus is to grow. There was an inhibitory factor present in the non-essential amino acids which masked the growth effect of tyrosine, but this was not pursued further.

TABLE II

GROWTH FACTORS REQUIRED BY *E. ashbyi* IN SCHOPFER'S MINIMAL MEDIUM ADJUSTED TO DIFFERENT pH VALUES

Compounds added	Amount of each compound per 5 ml. of medium	pH range at which growth occurs
1. Yeast extract	5 mg.	5.0–7.8
2. Methionine, histidine, and tyrosine	0.1 mg.	5.0–7.6
3. Methionine and histidine	0.1 mg.	5.5–7.6
4. Methionine	0.1 mg.	5.8–7.6
5. None	——	6.2–7.2
6. Minimal medium minus biotin, inositol, and thiamin	——	None

The report of Schopfer and Guilloud (**12**) that the addition of leucine or leucine and arginine would permit the growth of *E. ashbyi* in a synthetic medium, whose pH after autoclaving would be approximately 5.0, could not be confirmed with the strain of fungus available in this laboratory. The exact levels of arginine, proline and glutamate which, according to Dulaney and Grutter, would support the growth of the fungus in a synthetic medium were not given, though the level must be high since it was stated to be over 0.1%. Various cultures of *E. ashbyi* were collected from other laboratories and tested for their growth factor requirement. The names of laboratories from which cultures were received were given earlier. The strain from the Lederle laboratories was the only one different from the culture available in this laboratory and used in the experiments reported in this paper. The Lederle strain could grow at pH 5.0 with the addition of only methionine to the synthetic medium.

Compounds influencing the production of riboflavin. The following work consisted mainly of feeding the fungus various compounds, active in either known or unknown functions in the cell,

which might influence riboflavin production. The same synthetic medium with the addition of methionine and the same methods, as given before, were used throughout this phase of the problem. All of the known growth factors were tested in the following large groups: B-vitamin solution, essential amino acids, non-essential amino acids, and hydrolyzed yeast nucleic acid. Data in TABLE III show that the essential amino acids increase production of riboflavin markedly.

TABLE III

EFFECT OF VARIOUS OTHER GROWTH FACTORS UPON PRODUCTION OF B_2 IN *E. ashbyi*

0.1 ml. of stock solution per 5 ml. of basal medium pH 5.8

Combination of growth factors	Amount of growth	Yield of B_2 in μg. per ml. of medium
Vitamin solution	++++	44 μg.
Essential amino acids	++++	150 μg.
Non-essential amino acids	++++	40 μg.
Hydrolysed yeast nucleic acid	++++	46 μg.
Control (medium plus .1 mg. methionine	++++	44 μg.
Control (1% peptone—Parke Davis Bacteriologic)	++++	196 μg.

The lack of effect by the other combinations might result from inhibition by one component of the mixture which masked any beneficial effect of the others.

From these results it was apparent that one or more of the essential amino acids played a large role, directly or indirectly, in the biosynthesis of riboflavin. In TABLE IV the effect of adding

TABLE IV

EFFECT OF INDIVIDUAL ESSENTIAL AMINO ACIDS UPON PRODUCTION OF B_2 IN *E. ashbyi*

0.1 mg. of amino acids per 5 ml. of basal medium plus methionine pH 5.8, grown 5 days

Essential amino acid	Amount of growth	Yield of B_2 in μg. per ml. of medium
L-valine	++++	23 μg.
DL-leucine	++++	25 μg.
L-histidine	++++	63 μg
L-arginine	+++	23 μg.
L-lysine	++++	24 μg.
DL-isoleucine	++++	24 μg.
DL-threonine	++++	22 μg.
L-tryptophane	+++	22 μg.
DL-phenylalanine	++++	25 μg.
Control (medium plus methionine)	++++	24 μg.

the amino acids individually to the basal medium plus methionine is shown. The addition of histidine more than doubled the riboflavin yield as compared with a medium containing methionine as the only essential amino acid added. The other amino acids gave yields comparable to the control.

The amounts of methionine alone and methionine plus histidine which gave the highest yields of the vitamin were determined. Dry weights of the fungus were taken at the same time to determine whether riboflavin production under the varying amounts of added amino acids was dependent upon or independent of the amount of growth of the fungus. The results are shown in TABLE V.

TABLE V

INFLUENCES OF INCREASING AMOUNTS OF METHIONINE AND HISTIDINE UPON DRY WEIGHT AND PRODUCTION OF RIBOFLAVIN OF *E. ashbyi*

pH 5.8

Amount of methionine and histidine per 20 ml. of medium	Dry weight of fungus	Yield of B_2 in μg. per ml. of medium
None	0	0
0.2 mg. methionine	13.6 mg.	2 μg.
0.4 mg. methionine	20.4 mg.	20 μg.
0.8 mg. methionine	24.0 mg.	44 μg.
1.2 mg. methionine	23.9 mg.	36 μg.
1.6 mg. methionine	20.4 mg.	24 μg.
0.2 mg. histidine plus 0.8 mg. methionine	24.3 mg.	86 μg.
0.4 mg. histidine plus 0.8 mg. methionine	23.8 mg.	110 μg.
0.8 mg. histidine plus 0.8 mg. methionine	24.4 mg.	146 μg.
1.2 mg. histidine plus 0.8 mg. methionine	21.9 mg.	142 μg.
1.6 mg. histidine plus 0.8 mg. methionine	22.3 mg.	140 μg.

It was clear from these results that methionine while increasing riboflavin production up to a certain point also increased the growth and thus the dry weight of the fungus. More histidine increased riboflavin production independently of any increase in dry weight.

It was found that the addition of 0.8 mg. of valine to 20 ml. of the basal medium containing the optimum amounts of methionine and histidine increased the yield of riboflavin slightly. The yield of the vitamin on the addition of these three amino acids equaled that on the addition of the essential amino acid mixture.

Some compounds used in the chemical synthesis [3] of riboflavin were added to the basal medium plus methionine and histidine to

[3] These compounds were kindly furnished by Merck & Company and Hoffman-LaRoche, Inc.

test for increased yields of this vitamin. If any of the compounds added were naturally occurring precursors, the fungus should be able to convert them to riboflavin. The results are presented in TABLE VI.

TABLE VI

EFFECT OF VARIOUS COMPOUNDS USED IN CHEMICAL SYNTHESIS OF B_2 UPON YIELD OF B_2 OF *E. ashbyi* GROWN IN MINIMAL MEDIUM PLUS METHIONINE AND HISTIDINE

pH 5.8, grown 10 days

Compound added	Amount added per 20 ml. of medium	Yield of B_2 in μg. per ml. of medium
I. D-Ribose	1 mg.	201 μg.
II. Alloxan monohydrate	1 mg.	236 μg.
III. Tetraacetyl-D-ribose	1 mg.	86 μg.
IV. o-Phenylenediamine	1 mg.	26 μg.
V. 1-(D-Ribitylamino)-3, 4-dimethylbenzene	1 mg.	220 μg.
VI. 1-(D-Ribitylamino)-2-amino-4, 5-dimethylbenzene hydrochloride	1 mg.	215 μg.
VII. 1-(D-Ribitylamino)-2-phenylazo-4, 5-dimethylbenzene	1 mg.	218 μg.
II. plus VI	1 mg. of each compound	239 μg.
Minimal medium plus methionine and histidine	0	223 μg.

There was no increase in the production of riboflavin from the addition of any of the compounds. The decreased production of the vitamins on the addition of tetraacetyl-D-ribose and D-o-phenylenediamine was due, at least in part, to the inhibition of growth of *E. ashbyi*. Unless the rate of conversion was very low, there seemed to be little possibility that any of the compounds were precursors.

DISCUSSION

The varying needs of the fungus for amino acids were probably due to their ability to produce these substances only in certain pH ranges. Mutation involving sensitivity to pH has been reported in *Neurospora* (**15**). For example, *E. ashbyi* could manufacture the necessary methionine at pH 6.4 but at pH 5.8 it could not synthesize this amino acid and so methionine had to be supplied in the culture medium. The ability of the fungus to adapt and be able to grow with no addition of growth factors at lower pH's was not tested. This type of possible adaptation due to the various

methods of maintaining the viability of the cultures might explain the difference in growth factor requirements reported from different laboratories.

Why *E. ashbyi* can use homocysteine, cysteine, or sodium thioglycollate in place of methionine, but not homocystine or cystine, is not known. Two possibilities are suggested: either it might be due to difference in oxidation-reduction potentials of the compounds or to the fact that the fungus lacks the enzyme necessary for splitting the S to S bond in homocystine and cystine.

Any interpretation of the data given must attempt to explain the marked increase in riboflavin production by the addition of the amino acids, particularly histidine. The effect of methionine on riboflavin production is due to the increased growth of the fungus with increased riboflavin production following as a natural consequence. Histidine, on the other hand, gives increased riboflavin yields not correlated with the increased growth of *E. ashbyi*.

One explanation of the increase in riboflavin related to the addition of histidine might be that the histidine molecule or a portion such as the imidazole ring is incorporated as a part of the isoalloxazine nucleus of the vitamin. Imidazole, a constituent of the histidine molecule, was fed to the fungus but there was no increase of riboflavin. However, there is no evidence that imidazole alone can be actively metabolized by microorganisms. Another explanation for the increased production of riboflavin when histidine was added might be its role as a component of a protein molecule or enzyme which catalyses a step or steps in the biosynthesis of riboflavin. As soon as a balance of amino acids was present which was necessary for full protein synthesis of this particular type of enzyme, the further addition of histidine was without effect. The increase correlated with the addition of histidine is approximately 25 μg. of riboflavin for 1 μg. of the amino acid added. If the histidine were going into the isoalloxazine molecule, the increase should be more of the nature of two to one, unless both hypotheses were true and were operating simultaneously. The increase due to valine possibly could be explained by its role of completing the proper protein complement for best riboflavin production.

SUMMARY

1. The growth factor requirements of the experimental strain of *E. ashbyi* were found to be closely associated with variations in the pH of the medium. Only certain amino acids were required at certain pH levels and there was a pH range where no additional factors were required.

2. Methionine, histidine, and tyrosine were the amino acids required at the different pH ranges.

3. Histidine plays a very important role in the biosynthesis of riboflavin by this fungus.

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NOTES ON INDIAN USTILAGINEAE. IV

M. S. Pavgi and M. J. Thirumalachar

(with 27 figures)

Since the publication of the previous notes (**4**, **2**) further collections of smuts from different localities have been made and studied. Chlamydospore germination and artificial culture have been carried out in few cases. A brief account of these studies is presented here.

(1) *Sphacelotheca bicornis* (P. Henn.) Zundel, Mycologia **22**: 140. 1930.

Hab. in the inflorescence of *Cymbopogon flexuosus* (Steud.) Watson, leg. M. J. Narasimhan, Nandi Hills, Mysore, 18–2–1947. (Figs. 1–4.)

Sphacelotheca bicornis has been described on *Andropogon bicornis* from Brazil, the type locality, and has so far not been reported to occur in any other place. The smut incites remarkable witches-broom-like malformations of the inflorescence, formed by the grouping together of the sori at one point like a verticel (Viegas, 1945). The sori are at first covered by a reddish-brown false membrane, which flakes away at maturity, revealing the dark-brown spore mass. Mature spores are reddish-brown, subglobose to irregularly polyhedral, medium thick-walled, verrucose, 7.5–12 μ in diameter, with a mean of 8.4 μ. The spores germinated readily when placed on drops of water on slides and incubated in moist chambers for 48 hours. The sporidia are produced laterally and terminally on septate promycelia. The present record of the smut outside the type locality on a new host is interesting.

(2) *Sphacelotheca Arundinellae* (Bref.) Mundkur, Trans. Brit. Myc. Soc. **23**: 113. 1939.

Hab. in the ovaries of *Arundinella setosa* Trim., leg. M. J. Thirumalachar, Nandi Hills, Mysore, 8–3–1945. (Fig. 5–8.)

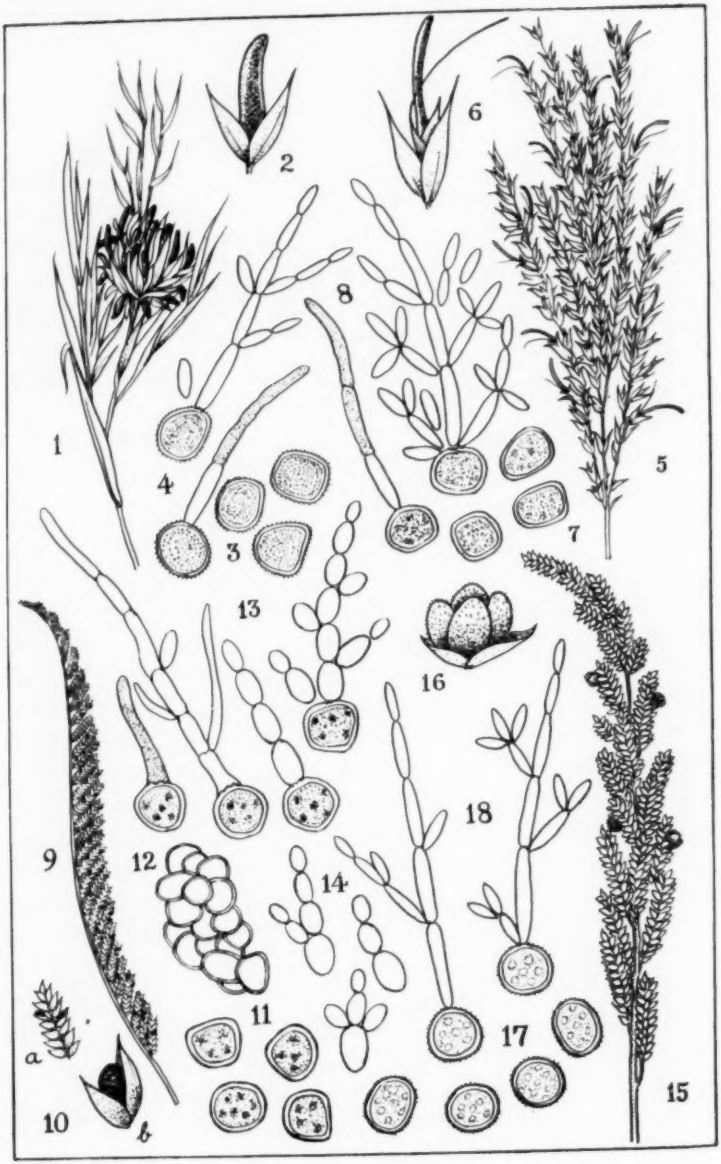
The smut, which was first described by Brefeld under the name *Ustilago Arundinellae* Bref., was based on a single collection made by Cunningham and has not been collected since then. The sori are 2 to 3 mm. long, protruding out of the glumes. The chlamydospores germinated readily within 24 hours when mounted in drops of water and incubated at room temperature in moist chamber. The promycelia are septate, bearing both lateral and terminal sporidia. Germinating chlamydospores were transferred aseptically to potato dextrose agar, producing a sporidial type of growth and delicate hyphal strands. In old cultures the production of chlamydospores was observed appearing as small black pin-heads. The chlamydospores produced in artificial cultures were similar to those formed naturally on the host.

(3) *Sorosporium Turneri* McAlpine, Smuts of Australia 185. 1910.

Hab. in the ovaries of *Eragrostis* sp., Majhgawan, Uttar Pradesh, leg. M. S. Pavgi, 18–10–1950. (FIGS. 9–14.)

In the spikelets of *Eragrostis* sp. an ovaricolous smut was collected which on examination proved to be identical with *Sorosporium Turneri* reported by McAlpine from Australia. The infection is very inconspicuous and is masked due to association with *Helminthosporium Ravenelii* Curt. & Berk. which formed a sooty covering on the spikelets. Only scattered ovaries in the inflorescence are smutted. Examination of the immature sori reveals that the spore balls are firm at the beginning, compactly grouped, and measure 70–110 μ in diameter. In mature sori, however, the spore balls are less evident and the spores become separated. Mature spores are olive-brown, subglobose to polygonal, due to lateral compression within the spore ball, smooth, measuring 6.5–10.5 μ in diameter with a mean of 8.8 μ.

The chlamydospores were germinated and stained by the method suggested by the writers (**5**). The promycelium is septate, bearing lateral and terminal sporidia characteristic of the Ustilaginaceae. Under low moisture conditions the promycelial cells are readily separated, becoming moniliform, budding off sprout cells. There has been no previous record of this smut in India (**1**).



FIGS. 1–18.

(4) *Ustilago Panici-frumentacei* Bref. Unters. Gesammt. Mykol. **12**: 103. 1896.

Hab. in the ovaries of *Echinochloa Crus-galli* Beauv., Benares, leg. M. S. Pavgi, 20–8–1950. (FIGS. 15–18.)

This smut has previously been reported on *Echinochloa frumentacea* (Roxb.) Link from several places in India. The infected ovary mostly appears as a four-lobed structure enclosing an abundant spore mass. The chlamydospores germinate by formation of a dendritic promycelium bearing lateral and terminal sporidia in rapid succession.

(5) **Tilletia setaricola** sp. nov.

Sori in ovariis, quorum nonnulla tantum pereunt; ovaria infecta bullata, membrana crassa atque brunnea operta, quae rumpitur expositis sporis. Sporae maturae globosae, fusce cinnamomeo-brunneae, absque membrana hyalina, 17.5–25 μ in diam., medietate 21 μ. Episporium crassum, reticulationibus 5- vel 6-angularibus ornatum, quae 5 μ diam. obtinent. Sporae steriles nullae.

Hab. in ovariis *Setariae intermediae* R. & S.

Sori in the ovaries, only a few in the panicle destroyed, infected ovaries bullate, covered by a thick brownish membrane which ruptures, exposing the spores. Mature spores globose, dark cinnamon-brown, without hyaline membrane, 17.5–25 μ in diameter with a mean of 21 μ. Epispore thick, with 5–6-angled reticulations which are 5 μ in diameter. Sterile cells absent.

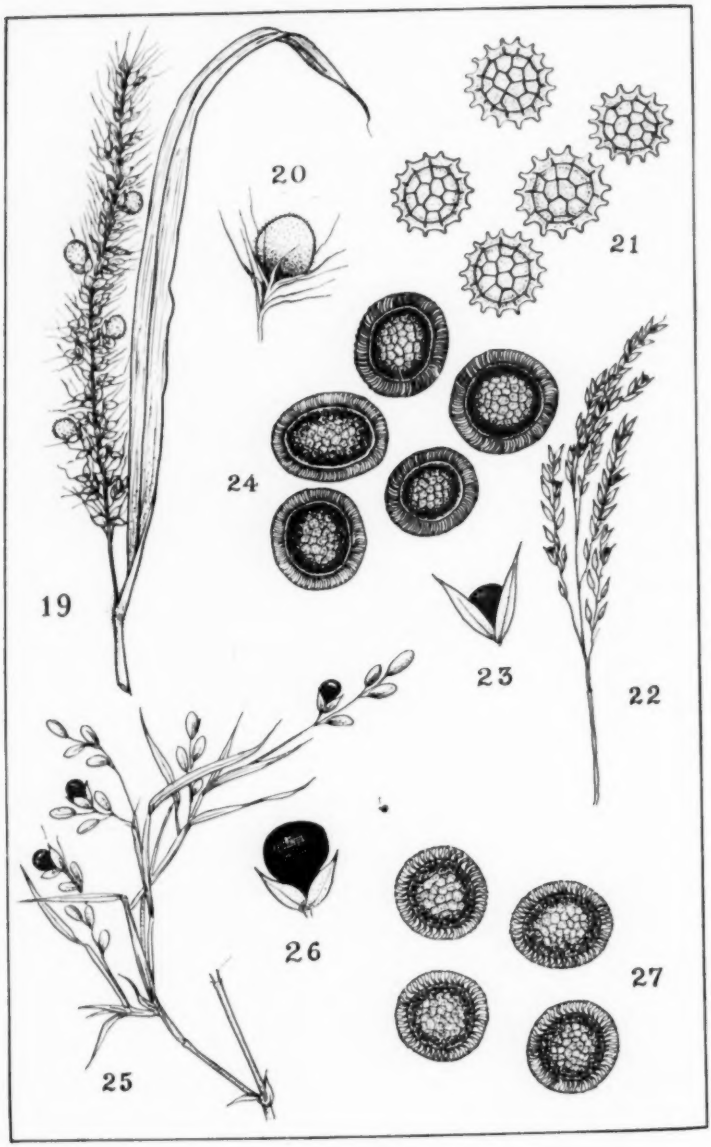
Hab. in the ovaries of *Setaria intermedia* R. & S., Benares, leg. M. S. Pavgi, 15–9–1950. (FIGS. 19–21.)

FIGS. 1–4. *Sphacelotheca bicornis*. 1. Smutted inflorescence of *Cymbopogon flexuosus*. ½ nat. size. 2. Sorus enlarged, × 2.5. 3. Chlamydospores, × 750. 4. Germination stages, × 750.

FIGS. 5–8. *Sphacelotheca Arundinellae*. 5. Smutted spikelets in the inflorescence, ½ nat. size. 6. Enlarged sorus, × 2.5. 7. Chlamydospores, × 750. 8. Germination stages, × 750.

FIGS. 9–14. *Sorosporium Turneri*. 9. Showing infected ovaries in the panicle, ½ nat. size. 10, a, b. Enlarged views of the sori. 11. Chlamydospores, × 750. 12. Spore ball, × 550. 13, 14. Germinating chlamydospores, × 750.

FIGS. 15–18. *Ustilago Panici-frumentacei*. 15. Showing infected ovaries in the panicle, ½ nat. size. 16. Enlarged view of sorus, × 3. 17. Chlamydospores, × 750. 18. Germinating spores, × 750.



FIGS. 19–27.

Collections of this interesting smut were made in a single clump of the grass growing in the vicinity of Benares Hindu University campus. The infected ovaries were several times larger than the healthy ones. Comparative studies indicated that the smut was different from *Tilletia echinosperma* Ainsworth (= *Ustilago heterospora* P. Henn.) on *Setaria sphacelata* Stapf. & C. E. Hubb. in East Africa and *Tilletia Setariae* Ling on *Setaria lutescens* from China. In *T. echinosperma*, authentic specimens of which were available for study, the chlamydospores were 13–16 μ in diameter, covered with blunt spines and possessing sterile cells of two sizes. In *T. Setariae* Ling, the spores are opaque, 22–33 μ in diameter and covered with truncate scale-like projections. Both these smuts are different from *T. setaricola* which possesses a reticulate epispore.

(6) *Tilletia pulcherrima* Ell. & Gall. var. **brachiariae** var. nov.

Varietati typicae similis, sed sporis praedita paulo minoribus, 19–26 μ in diam., medietate 21.5 μ.

Hab. *Brachiariae distachyae* Stapf.

Similar to *Tilletia pulcherrima* Ell. & Gall. but with spores which are smaller in size, 19–26 μ, with a mean of 21.5 μ.

Hab. in the ovaries of *Brachiaria distachya* Stapf., Benares, leg. M. S. Pavgi, 14–9–1950 (type), and in the ovaries of *Eriochloa procera* Hubb., Benares, leg. M. S. Pavgi, 20–9–1950.

The same smut species was found attacking the ovaries of *Brachiaria distachya* and *Eriochloa procera*, which are segregates of the genus *Panicum*. The smut infected only few ovaries in the panicle, the sori being greenish-black and protruding slightly out of the glumes, 3–4 mm. long and 2–3 mm. broad. At maturity the black spore mass was exposed by the rupturing of the outer tough membrane. The mature spores were reddish-brown to opaque, subglobose to spherical, with evident hyaline membrane and

FIGS. 19–21. *Tilletia Setaricola*. 19. Smutted inflorescence of *Setaria intermedia*, nat. size. 20. Sorus enlarged, × 3. 21. Chlamydospores, × 500.

FIGS. 22–27. *Tilletia pulcherrima* var. *Brachiariae*. 22. Smutted inflorescence of *Eriochloa procera*, nat. size. 23. Sorus, × 4. 24. Chlamydospores, × 750. 25. Smutted spikelets of *Brachiaria distachya*, ½ nat. size. 26. Enlarged view of sorus, × 3. 27. Chlamydospores, × 750.

acute to truncate scale-like projections in sectional view. The spores measured 19–26 μ in diameter with a mean of 21.5 μ. The sterile cells were numerous, hyaline, very thick-walled, smaller than the spores, mostly 9–22 μ.

In morphological characters the smut is identical with *Tilletia pulcherrima* Ell. & Gall. described on species of *Panicum* in the United States. Spore measurements taken from authentic specimens of *T. pulcherrima* showed a range of 18–30 μ, with a mean of 25 μ. The variety *Brachiariae* described by us has slightly smaller spores and a smaller mean than *T. pulcherrima*.

In conclusion we wish to express our grateful thanks to Rev. Father Dr. H. Santapau, Professor of Botany, St. Xavier's College, Bombay, for kindly giving the Latin diagnoses of the new species and variety.

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DIFFERENCES IN SPORE SURFACE MARKINGS OF THREE PINE RUSTS, AS SHOWN BY THE ELECTRON MICROSCOPE

JAMES L. MIELKE[1] AND GEORGE W. COCHRAN[2]

(WITH 1 FIGURE)

The three pine-inhabiting rusts, *Cronartium harknessii* (Moore) Meinecke, *C. filamentosum* Hedgc., and *C. stalactiforme* Arth. & Kern., were originally described as distinct species. Later, successful cultures of the three were reported on Indian paintbrush (*Castilleja* spp.) on which the spore stages produced were said to be indistinguishable. This led some workers to regard them as a single species, which Arthur (**2**) designated as *Cronartium coleosporioides* (Diet. & Holw.) Arth., in spite of differences among the three in their effects on the pine hosts and particularly in the structure of their respective aecia. When Arthur (**1**) adopted this view, however, he conceded that they might be treated as distinct species or as varieties.

Arthur (**2**, p. 30), in a later publication in which the three species in question were again included under the name *Cronartium coleosporioides*, briefly summarized the pertinent literature, as follows:

"The aecia of this species assume three fairly distinguishable forms, which have been shown by cultures to produce uredia and telia of identical appearance on the same species of *Castilleja*. Hedgcock (Phytop. **2**: 176. 1912) made successful cultures with *P. filamentosum*. Meinecke (Phytop. **3**: 167. 1913), Hedgcock and Long (same, p. 250) and Weir and Hubert (Phytop. **7**: 106. 1917) used aecia of *P. stalactiforme*. Cultures in the open of

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P. harknessii were made by Bethel in 1909–13 (Phytop. **8**: 317. 1918), and in 1915 in the greenhouse by Weir and Hubert (Jour. Agric. Res. **5**: 782. 1915), and again in 1916 (Phytop. **7**: 107. 1917). The three forms may be treated as distinct species (Arthur, Bull. Torrey Club **49**: 194. 1922; N. Am. Flora **7**: 695. 1925), or as varieties under the names: *C. coleosporioides filamentosum, C. c. stalactiforme,* and *C. c. harknessii.*"

Based on the results of studies made later than those cited by Arthur in the preceding paragraph, both Hedgcock (**4**) and Weir (**6**) concluded that the three rusts were distinct species. Mielke (**5**) concurs in this treatment as a result of investigations involving mainly *Cronartium filamentosum.* It appears that most individuals interested in these rusts have experienced difficulty in differentiating between *C. filamentosum* and *C. stalactiforme.*

Arthur (**2**, p. 29) described the aeciospores of the inclusively interpreted species, *Cronartium coleosporioides,* as "coarsely verrucose," without indicating any differences in the surface characteristics of these spores for the three rust forms included in it. Previously, however, Arthur and Kern (**3**) described the spores of both *C. filamentosum* and *C. stalactiforme* as "moderately verrucose" and those of *C. harknessii* as "finely verrucose." Weir (**6**) described the aeciospores of *C. filamentosum* as "evenly verrucose, processes short" and of *C. stalactiforme* as "moderately verrucose, processes frequently flattened." In order to determine whether differences could be noted at higher magnifications than those provided by the ordinary microscope, representative aeciospores of the three forms were examined under the electron microscope.

SPORE MATERIAL EXAMINED

For each rust, examinations were made of aeciospores from two collections obtained at widely separated localities as follows: *Cronartium filamentosum* on *Pinus ponderosa* var. *scopulorum* Engelm., from Utah and the type locality in southern Arizona; *C. stalactiforme* on *P. contorta* Dougl., Idaho and California; and *C. harknessii* on *P. contorta,* Idaho, and *P. ponderosa* Laws., California.

In preparing the material for examination, dry aeciospores were dusted onto specimen screens covered with formvar membranes. Mounted in this manner, the spores were then studied with a

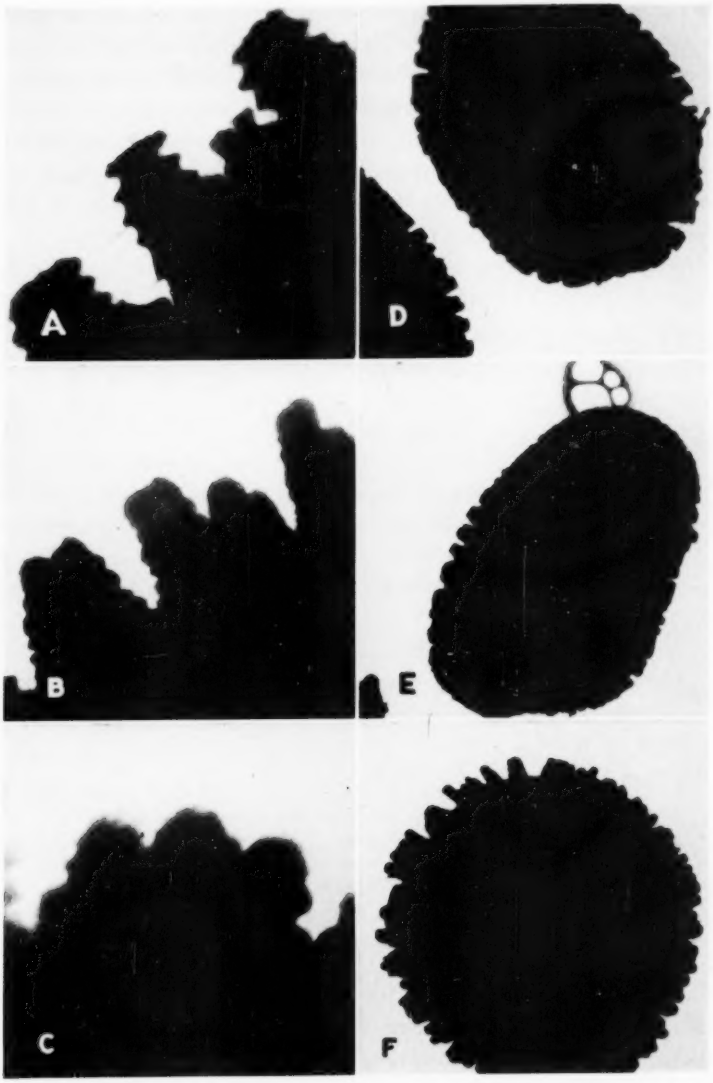

FIG. 1. Electron micrographs, showing spore characters. A, B and C. Processes on the surface of aeciospores of *Cronartium filamentosum*, *C. stalactiforme* and *C. harknessii*, respectively, × 25,000. D, E and F. Silhouettes of the aeciospores of *C. filamentosum*, *C. stalactiforme* and *C. harknessii*, respectively, × 1,500. Because of the position in which these spores were lying when photographed, the smooth area, generally found near an end or along one side, is not shown.

R.C.A., E.M.U. 2B electron microscope equipped with a bias gun and an extended range lens. Hundreds of spores of each species were examined and approximately 50 of each were photographed. The spores and portions thereof illustrated herein (FIG. 1) are characteristic for each species. It should be pointed out that since the electrons will not pass through the spores the resulting images are silhouettes. Thus, only the surface details of the spores at an equator can be viewed and photographed.

RESULTS

As a result of the study of aeciospore surfaces, differences have been found in the shape of the processes for each of the three rusts, also in the general outline of the spores. The principal differences in the processes are as follows:

Cronartium filamentosum (FIG. 1, A)

Processes usually prominently annulated; annulations generally acutely serrated in outline.

Tops of processes generally flattish, rather finely and irregularly serrated.

Cronartium stalactiforme (FIG. 1, B)

Processes usually prominently annulated; annulations generally obtusely serrated in outline.

Tops of processes generally roundish, more coarsely serrated than C. *filamentosum*.

Cronartium harknessii (FIG. 1, C)

Processes infrequently annulated; annulations obtuse when present.

Tops of processes irregularly rounded, generally more coarsely serrated than *C. stalactiforme*.

Silhouettes of *Cronartium harknessii* aeciospores (FIG. 1, F) show a much more broken or roughened outline than do these spores of either *C. filamentosum* (FIG. 1, D) or *C. stalactiforme* (FIG. 1, E). Spores of the latter two fungi are fairly similar in their outline appearance at the magnification here illustrated, which is about 1,500 diameters. Also at this magnification, some of the processes may appear similar in shape. This results mainly be-

cause of the generally dense population of the processes, which does not always make possible clear differentiation of the individuals. It is not until much higher magnifications are employed and individual processes examined, however, that the differences among them become apparent. A magnification of × 25,000 clearly illustrates these differences. For the three rusts, no uniformity in size of the processes was found.

The differences that we have found to exist among the surface configurations of the respective aeciospores are regarded as supporting the view that *Cronartium filamentosum, C. stalactiforme,* and *C. harknessii* should be treated as distinct species. It would have been desirable to have studied also the perfect stage of each of the rusts. However, authentic material for this purpose was not available and there is some question whether it exists for typical *C. filamentosum* because of the absence of this stage on reported alternate hosts in areas where the rust is prevalent on pines (**5**) and because of the failure of recent attempts to produce infection on plants of *Castilleja linariaefolia* Benth., with aeciospores of this rust.

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CULTURE STUDIES IN THE GENERA PLEOSPORA, CLATHROSPORA, AND LEPTOSPHAERIA

EMORY G. SIMMONS

(WITH 13 FIGURES)

The classification of Pyrenomycetes is at present in a chaotic condition. Any natural and usable classification depends upon a more detailed knowledge of the life cycles, development, and variability of a much larger number of species than has been studied. Such information is not always easy to obtain because of technical difficulties involved in making proper observations and in obtaining significant data.

The systematic arrangement of families and, to some extent, of genera in the Pyrenomycetes is based upon the type of perithecial development. Delimitation of species is established primarily on the morphology of the perithecial stage, often on data derived from a very limited number of collections. It is desirable, therefore, to extend the procedure of species circumscription to reflect: (a) a knowledge of the pattern of variation of the natural population and (b) a knowledge of the range and the mechanism of variation in both the ascus and the conidial stages and of the correlation of these two stages.

The determination of these factors is hampered by certain unsolved problems:

(1) work on the cytology and the developmental morphology of the perfect fruiting body has been retarded by the difficulty of obtaining perithecia in culture and by the technical difficulties of staining and of interpreting the complex hyphal structures within the perithecia;

(2) the determination of genetic behavior and of the cause and mechanism of variation in these organisms also depends upon the routine formation of perithecia in culture under controlled conditions;

(3) the knowledge of variability of imperfect stages is not on a firm basis because of the lack of known genetic connections with ascus stages and the lack of comparative studies of variation in pure strains.

The range of these problems is so great that it soon became obvious that no immediately complete and final study could be made of any one of them. There was made, rather, a preliminary survey of this field by a cultural study of several species in three genera of the Pleosporaceae with more detailed excursions into several problems which presented possibilities of positive results.

The first line of investigation was concerned with the behavior of various strains and species in the formation of conidial stages and with the variation of conidia within and between isolates. These problems were studied in some detail and are reported upon in the present paper.

Secondly, because of the importance of production of perithecia in culture for studies in cytology, genetics, or ascospore variation, special attention was given to this problem. In addition, a preliminary study was made of the variation in ascospore length in single pure cultures of three different perithecial strains. A second paper in preparation is designed to present culture data and derivative information on production of mature fruiting bodies of these fungi.

The Pleosporaceae have a pseudosphaeriaceous type of development and produce solitary fruiting bodies beneath the surface of stems and leaves of herbaceous and woody plants. The genera considered here differ in their types of ascospores, as follows:

Pleospora—ascospores with transverse and longitudinal septa; spores not flattened.

Clathrospora—ascospores with transverse and longitudinal septa; spores flattened.

Leptosphaeria—ascospores with transverse septa only; spores not flattened.

In addition to the ascospores borne in sac-like asci in the fruiting body, species of these genera also may have conidial stages of different sorts borne free upon the mycelium or within fruiting structures.

Mycological literature contains numerous statements concerning ontogenetic connections between species of *Pleospora* and of *Leptosphaeria* and members of the Fungi Imperfecti. Usually these assertions are based on observations that a perithecial stage and an imperfect stage are closely associated in nature; only infrequently are conclusions concerning such connections based on experimental studies in which one stage is seen to develop from pure cultures of spores of the other stage.

A classic example of the confusion raised over the ontogenetic connection of imperfect stages with a perithecial stage is the history of studies made on *Pleospora herbarum* (Pers.) Rab. Tulasne (**1863**) listed no less than sixteen binomials in his discussion of the pleomorphism of this species, including members of the form-families Sphaerioidaceae, Moniliaceae, Dematiaceae, and Tuberculariaceae. This list was augmented and depleted in turn through the years following studies by several investigators (Fuckel, **1870**; Hallier, **1869**; Gibelli and Griffini, **1874**; Bauke, **1877**; Kohl, **1883**; and Mattirolo, **1888**). Using artificial culture techniques, Miyabe (**1889**) conclusively established the insertion of a single imperfect stage, *Macrosporium parasiticum* Thüm. [= *Stemphylium botryosum* Wallr.], in the life cycle of *P. herbarum*. Since the time of Miyabe, his conclusions have been affirmed by a number of other investigators.

No other single species of either *Pleospora* or *Leptosphaeria* has received such detailed attention, but different authors have indicated conidial connections of at least 45 named species of *Pleospora* and 50 named species of *Leptosphaeria*. Most of these connections, as has been stated, have been based on field observations, but a few (not more than 10 in all) have been worked out in controlled culture and have become established in the literature on pleomorphism.

Aside from phytopathological applications the knowledge concerning these conidial connections has not been employed to any great extent in mycological work. The hope that a correlation of imperfect with perfect stages would aid in the construction of a natural system of taxonomy has been hindered by the scarcity of reliable data. Moreover, the present limited understanding of

what constitutes a genetic entity in the Fungi Imperfecti prevents the resolution of this problem from a systematic standpoint.

MATERIALS AND METHODS

A. *Collections Used.*

Identified specimens used in this study are listed at the end of the paper. They will be referred to by the study numbers (1–43) of that listing.

B. *Media and Plant Substrata Used.*

The principal medium employed in the isolation, maintenance, and comparison of all isolates was Leonian's agar (**1924**), and since data in the literature do not indicate that the type of organisms being studied here must grow on any specific medium in order to produce perithecial stages, it also was used for this purpose.

Some isolates also were grown on other types of media so that the influence of different substrata upon vegetative growth, conidial production, and perithecial formation could be studied. These media and plant substrata, which are described below, were sterilized by means of autoclaving with steam at 121° C. for 20 minutes.

Leonian's medium: KH_2PO_4, 1.25 g.; $MgSO_4 \cdot 7H_2O$, 0.625 g.; peptone, 0.625 g.; maltose, 6.25 g.; malt extract, 6.25 g.; distilled water, 1000 ml.

Leonian's agar: Leonian's medium solidified by the addition of 20.0 g. agar to 1000 ml. of the liquid medium.

Oatmeal agar: 40 g. rolled oats, ground, then cooked while being stirred in small amount (200 ml.) of water; added, while hot, to 800 ml. of distilled water in which had been dissolved 20.0 g. agar.

Mineral-peptone agar: KH_2PO_4, 1.25 g.; $MgSO_4 \cdot 7H_2O$, 0.625 g.; peptone, 0.625 g.; agar, 20.0 g.; distilled water, 1000 ml.

Mineral agar: KH_2PO_4, 1.25 g.; $MgSO_4 \cdot 7H_2O$, 0.625 g.; agar, 20.0 g.; distilled water, 1000 ml.

Malt agar: malt extract, 20.0 g.; agar, 20.0 g.; distilled water, 1000 ml.

Plant stem substratum: 12 cm. lengths of dead stems of *Chrysanthemum* sp.; autoclaved in test tubes 17.5 cm. long to which had been added 5 ml. of distilled water.

Filter paper cones: a filter paper disc was converted into a cone; the cone was placed on its base and its vertex was then depressed so that the truncated base of the original cone supported a small paper-walled well. This cone was placed in a small glass capsule-dish to which any desired liquid medium was added before autoclaving.

C. *Isolation Methods.*

In attempting an expansion of the criteria for the identification and the circumscription of species of *Pleospora, Clathrospora,* and *Leptosphaeria* it is considered essential that cultures of undoubted purity be compared.

Using aseptic techniques, perithecia of identified specimens were removed from the host tissues and were crushed in distilled water. Ascospores were then transferred to hanging drops of sterile tap water in concavity slides. These preparations were allowed to remain at room temperature (22°–26° C.) until germination occurred or, in the instance of spores which revealed no evidence of germination, for at least two weeks. Since most of the spores of the majority of preparations showed evidence of germination within 24 hours, the time period of two weeks was considered ample for the expression of spore viability under the conditions imposed. In the case of some perithecia which did not yield germinating spores in water, spore transfers were made to Leonian's medium or to a decoction of *Aster* stems in concavity slides. In some cases germination was induced by this treatment. Camera lucida drawings were made of spores in different stages of germination in the drop cultures. Subsequently, individual germinated ascospores were isolated manually with the aid of fine needles and were transferred to Petri plates containing Leonian's agar. In some instances when single-spore isolates failed to become established on this medium, single asci with germinated spores or groups of germinated spores of undoubted origin were used in obtaining cultures. Careful microscopic checks were made in every case in order that the purity of the different isolates might be insured. After a length of time sufficient for the formation of well-established colonies by the different isolates which survived on Leonian's agar, subcul-

tures for further study were initiated on new or different artificial media and on sterile stems.

CULTURE STUDIES: GENERAL DATA

It is a common observation of many collectors that the types of fungi being considered here often produce mature perithecia in nature after overwintering on dead stems or leaves. Because of this behavior, preliminary test-cultures usually were allowed to remain at room temperature for periods of one to fifteen days, after which they were removed to a cold chamber at 5° C. It was found that under these conditions *Clathrospora diplospora* (Coll. No. 33) would develop perithecial primordia and, eventually, mature perithecia. Using this evidence of the effect of prolonged cold treatment upon the formation of perithecia, subsequent cultures of all isolates were treated in a similar manner. In most instances an initial period of growth at room temperature was permitted until the individual inoculates had formed well-established colonies.

In TABLE I are summarized the general results of this treatment of all species used in this study. The results indicated reveal the following information:

1. Ascospores from the perithecia of 14 of the 43 collections gave no evidence of germination. It is possible that the age of a specific collection of fungus may determine whether or not the perithecia present will yield viable ascospores. In *Pleospora asymmetrica*, *Clathrospora diplospora*, and *Leptosphaeria heterospora* the oldest specimens did not yield viable spores, whereas the younger ones did. Such age-viability correlation, however, was not found in all species. In *P. ambigua*, *P. helvetica*, and *P. rainierensis* viable ascospores were obtained from collections equally as old or older than similar specimens in which spores were not viable.

2. In 17 of the remaining 29 collections ascospores from the perithecia germinated in drops of liquid media but did not become established when transferred to agar media. It is possible that this failure was due to mechanical injury to the young mycelium or to drying. However, it should be noted that when spores of two or more collections of a single species were germinated, the young mycelia, when transferred, either all grew or all died. This

TABLE I

SUMMARY OF CULTURAL RESULTS FOR ALL ISOLATES OF SPECIES OF *Pleospora*, *Clathrospora*, AND *Leptosphaeria*

Collection number	Species name	Age in months	No germination	Germinated but not established	Established in culture	Conidia produced	Perithecia produced
1	*P. ambigua*	75	X				
2	*P. ambigua*	16			X		
3	*P. ambigua*	5			X		X
4	*P. ambigua*	5	X				
5	*P. ambigua*	3			X		
6	*P. ambigua*	5	X				
7	*P. asymmetrica*	2		X			
8	*P. asymmetrica*	2		X			
9	*P. asymmetrica*	2		X			
10	*P. asymmetrica*	3	X				
11	*P. comata*	2½	X				
12	*P. helvetica*	3		X			
13	*P. helvetica*	4		X			
14	*P. helvetica*	4		X			
15	*P. helvetica*	2½		X			
16	*P. helvetica*	3		X			
17	*P. helvetica*	3	X				
18	*P. helvetica*	3		X			
19	*P. herbarum*	4	X				
20	*P. herbarum* var. *occidentalis*	3	X				
21	*P. infectoria*	(18)	(X)		X	X	
22	*P. laxa*	2½		X			
23	*P. njegusensis*	10½			X		X
24	*P. njegusensis*	5			X		
25	*P. oligasca*	10	X				
26	*P. richtophensis* var. *pallida*	5		X			
27	*P. richtophensis* var. *pallida*	2		X			
28	*P. rubicunda* var. *americana*	3		X			
29	*P. trichostoma*	½			X		X
30	*C. Cookei*	17	X				
31	*C. diplospora*	34	X				
32	*C. diplospora*	21			X		X
33	*C. diplospora*—I	4			X		X
	C. diplospora—II	4			X	X	
34	*C. Elynae*	14			X	X	
35	*C. permunda*	2½			X		
36	*P. rainierensis*	12	X				
37	*P. rainierensis*	14			X		X
38	*C. Simmonsii*	2½		X			
39	*C. Simmonsii*	2½		X			
40	*L. Erigerontis*	2½		X			
41	*L. filiformis*	3		X			
42	*L. heterospora*	4	X				
43	*L. heterospora*	1			X	X	

would indicate that, in the species which failed to grow, the nutritional or other environmental requirements of the organisms were not met by the conditions imposed upon them. (Early attempts at germination of spores on solid media were carried out, but this technique was discontinued when it was found that the incidence of contamination was high and that making critical observations for drawings was difficult.)

3. Three isolates from different species in the remaining 12 collections (in addition to the cultures of *P. infectoria* received from M. D. Whitehead) produced conidia in culture; six other isolates of ascospores from these 12 collections produced perithecial primordia and mature perithecia. In no case was it observed that a conidial strain also produced perithecia or that a perithecial strain also produced a conidial stage.

The 31 collections from which ascospores either did not germinate or did not become established on solid media will not be considered beyond their listing in TABLE I. The descriptions and behavior of isolates of ascospores from the 12 specimens which became established in culture (plus the *P. infectoria* cultures) are outlined in succeeding pages.

A. *Descriptions of Cultures.*

Pleospora ambigua (Berl. & Bres.) Wehm. (Collections Nos. 2, 3, 5; FIG. 1)

The three isolates of this species showed no differences in gross cultural characters. They all produced an abundant to heavy cottony, gray [1] to olive-gray aerial mycelium on both Leonian's and malt agar. An olive-gray to dark brown or black opaque growth occurred within the substratum.

Isolate No. 2 was derived from a single ascospore, whereas Nos. 3 and 5 were from two or more spores from single perithecia. These isolates were kept at room temperature for 14–20 days before removal to the cold room.

Conidia were not found in cultures of any of these isolates. Perithecia were formed in cultures of isolate No. 3 but not in

[1] Color values cited are used in a general sense in comparison of colonies of different species. They are not derived from a standard system of color names.

those of Nos. 2 and 5. Such a situation might be explained either by the presence of strains which do or do not give rise to perithecia or upon the basis of the presence of heterothallism, in which case isolate No. 3 might have included spores of both compatible strains whereas the spores of No. 5 would all have been of the same or incompatible strains. Which explanation is correct can be determined only by further single spore crosses.

Pleospora infectoria Fckl. (Collection No. 21)

The initial cultures of this species were received from M. D. Whitehead, Agricultural and Mechanical College of Texas. In correspondence Whitehead indicates that the cultures used here were derived from single ascospores. Subcultures were allowed to remain at room temperature for 20 days, after which they were removed to the cold chamber. On Leonian's agar a very minute amount of fine white mycelium was produced and became extended over the entire surface of the medium. Neither aerial hyphae nor conidia were observed on this substratum. In cultures on unsolidified Leonian's medium, however, a distinct submerged mat of olive-brown mycelium with an aerial covering of pale gray hyphae was produced. Conidia were very abundant in these liquid cultures. On oatmeal agar gray, rapidly spreading, dense cottony colonies with abundant conidia were produced. A heavy subsurface growth of black, opaque mycelium typically was formed. No evidence of the formation of perithecial primordia was seen in any culture of isolates of this species.

Pleospora njeguscnsis Bub. (Collections Nos. 23, 24; FIG. 2)

Both single-spore cultures and mass transfers of germinated spores were made from collection No. 23. No differences in development or in gross morphology were seen between single-spore and mass transfer cultures. These cultures were kept at room temperature for 12–15 days and then were transferred to 5° C. On Leonian's agar the rate of growth was decidedly slow, but within several weeks at cold room temperature growth of the colonies proceeded until the medium was covered and penetrated by distinctly radiate, dark olive-brown hyphae. A very loose cottony,

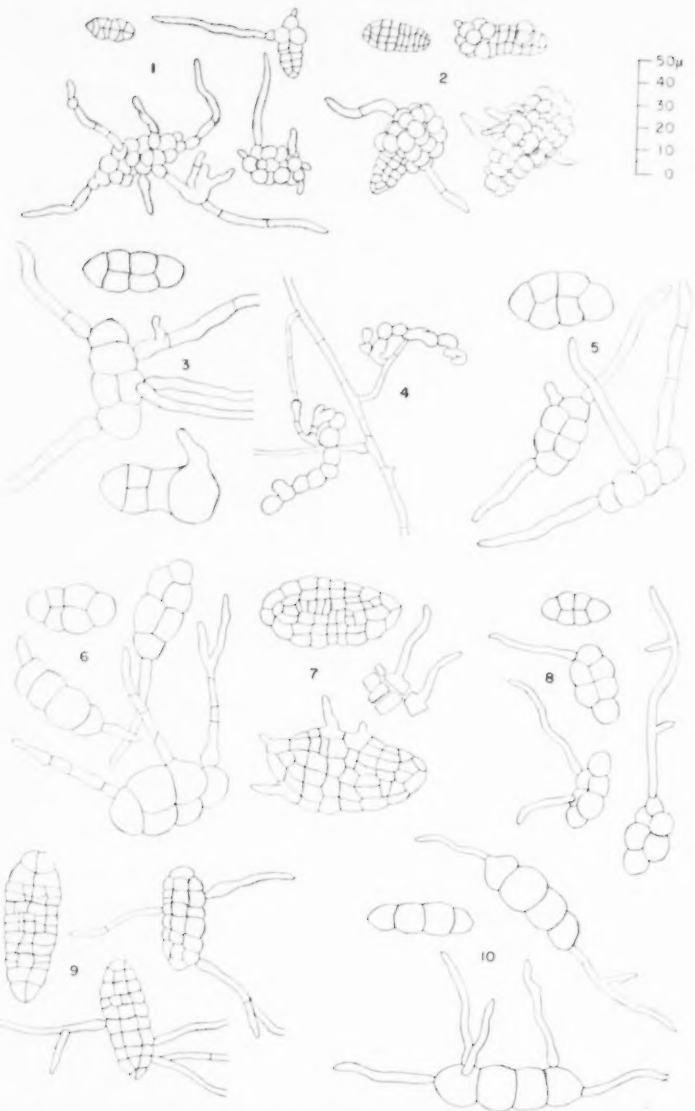
gray aerial mycelium was produced. Mature perithecia were produced in abundance in all these cultures within 16 weeks, but no conidia were found in any of them. This collection apparently contained a homothallic, non-conidial strain, for ascospores appeared in single-spore cultures.

Single-spore transfers from collection No. 24 to Leonian's agar did not survive, but mass transfers of germinated spores became established. These cultures were kept at room temperature for 21 days and then were transferred to the cold room. Within eight weeks a heavy growth consisting of a gray to dark olive-brown matted mycelium on and in the substratum and a loose cottony, light olive-gray to dark gray aerial mycelium with superficial, dense cottony patches of white hyphae was formed. Later the aerial mycelium disappeared from large areas of the colony, and the colony became heavy, black-brown, with a circumference of distinctly radiate, olive-brown subsurface mycelium. Neither conidia nor perithecial primordia were formed in any of these cultures. This strain, in contrast to that of No. 23, is either a heterothallic or a non-perithecial one. The probability is against a heterothallic condition, because no perithecia occurred even in mass-spore cultures. This indicates that certain strains fail to produce perithecia, at least under conditions which favor fruiting in other strains of the same species.

Pleospora trichostoma (Fr.) Ces. & de Not. (Collection No. 29; FIGS. 3 and 4)

A large number of single-spore and single-ascus isolates were made on Leonian's agar and on filter paper cones moistened with Leonian's medium. Among the cultures of single-spore isolates, two types of gross morphology of colonies became apparent by the third day.

Type I: Single-spore cultures yielded relatively scant, light gray mycelium and produced dark inflated cells (FIG. 4) in the substratum which, in the aggregate, became visible to the unaided eye by the third day; by the fourth day these structures appeared orange-brown to olive-green and occupied an area about 3 cm. in diameter in colonies which had reached a diameter of 4–5 cm. Cul-



FIGS. 1–10. Germinating ascospores and distinctive hyphae. 1. *Pleospora ambigua* (Berl. & Bres.) Wehm. 2. *Pleospora njegusensis* Bub. 3. *Pleospora trichostoma* (Fr.) Ces. & de Not. 4. Inflated hyphal cells produced on Leonian's agar by isolates of *Pleospora trichostoma*. 5. *Clathrospora*

tures derived from single asci exhibited these same growth characteristics.

Type II: Single-spore cultures yielded relatively heavy, light gray mycelium but did not produce discrete aggregates of inflated cells.

Differences between the types of gross morphology of the various isolates became less pronounced within a week because of the production in all cultures of a heavy gray to olive-gray aerial mycelium.

Cultures on Leonian's agar were kept at room temperature for 18 days before removal to the cold room. Cultures on moistened filter paper cones were kept at room temperature for 10 days, after which they were remoistened with sterile distilled water and were placed in the cold room. These isolates on filter paper produced a loose cobwebby, light to medium gray aerial mycelium. Perithecial primordia were produced in all cultures of both growth types, but conidia were not formed. By the end of 20 weeks mature ascospores were found in all cultures. A homothallic condition is indicated for this species.

Clathrospora diplospora (E. & E.) Wehm. In press (Collections Nos. 32, 33; FIGS. 5 and 6)

Transfers of single germinated spores of collection No. 32 to Leonian's agar were not successful; mass transfers of germinated spores were successful and subcultures were made from these isolates. Cultures on Leonian's agar were kept at room temperature for 12 days, by which time small pulvinate mycelial masses about 5 mm. in diameter were produced. During a subsequent period of six months at cold temperature, these colonies increased in diameter, producing an olive-brown to black, opaque growth in the center of each colony. The amount of mycelium outside this central area about 6 cm. in diameter was scarcely noticeable. A

diplospora (E. & E.) Wehm. in press (collection No. 32). 6. *Clathrospora diplospora* (E. & E.) Wehm. (collection No. 33). 7. *Clathrospora Elynae* Rab. 8. *Clathrospora permunda* (Cke.) Sacc. 9. *Pleospora rainierensis* Wehm. 10. *Leptosphaeria heterospora* (de Not.) Niessl.

light gray, loose cottony aerial mycelium was produced overall. Small, thin-walled perithecial primordia were formed in abundance in these colonies, but only a few comparatively large (up to 450 μ in diameter) individuals contained mature ascospores within eight months. Conidia were not observed at any time in these cultures.

Single-spore isolates of collection No. 33 (identified tentatively by Wehmeyer as a large-spored form of *Clathrospora diplospora*) readily became established on Leonian's agar. Cultures were left at room temperature seven to ten days before being removed to the cold room. Two distinct types of growth became evident in different single-spore cultures.

Type I: Within 30 days after being placed at the low temperature, colonies on Leonian's agar and on malt agar produced a dark olive-black mycelium which spread slowly over and beneath the surface of the medium. A very small amount of whitish aerial mycelium was evident, chiefly over the centers of the colonies. Numerous perithecial primordia were produced on the surface of or partially imbedded in the substrata. Mature ascospores were not found in these structures until after a period of about four months, at which time mature spores were present in abundance. Conidia were not observed in these cultures at any time. Since cultures of this type were derived from single spores, heterothallism is ruled out as a factor necessary for perithecium production.

Type II: Colonies on Leonian's agar and on malt agar spread rapidly and produced heavy, greenish-gray mats with a dense, white, cottony aerial growth. Concentric zonation was moderately marked in these cultures. Conidia were produced in great abundance, but perithecial primordia were not found in any culture of Type II within a period of 12 months at low temperature. In view of this fact, it would be practically impossible to establish the connection between this conidial strain and its perfect stage on the basis of perithecial production in the type of cultures used here. If a collection of this imperfect stage were obtained in nature, it could not be identified with its specific perithecial phase unless other physiologic or morphologic characters can be discovered for it.

Clathrospora Elynae Rab. (Collection No. 34; FIG. 7)

Transfers of germinated single spores became established on Leonian's agar. Subcultures were left at room temperature about seven days before being transferred to the cold room. Heavy, greenish-black colonies with a scant, whitish, cottony aerial mycelium were produced. Concentric zonation was marked; conidia were produced in abundance. Perithecial primordia were not found in any culture of this species within a period of 12 months at cold room temperature.

Clathrospora permunda (Cke.) Sacc. (Collection No. 35; FIG. 8)

Establishment of single-spore isolates on Leonian's agar was not achieved, but successful mass-transfers of germinated spores were made. Cultures were maintained at room temperature for 14–20 days and then were removed to the cold room. Rate of growth of the colonies was very slow. Colonies about 10 mm. in diameter and consisting of a medium brown, radiate mycelium with a white, cobwebby aerial covering were produced at room temperature within two to three weeks. After removal to the low temperature the darkened surface and subsurface mycelium did not advance more than a few millimeters in three months. Within the same period of time the light surface and aerial mycelium spread widely over the substratum but became obscure by the end of this time. Neither perithecial primordia nor conidia were found in these cultures at any time within a period of ten months.

Pleospora rainierensis Wehm. (Collection No. 37; FIG. 9)

Single-spore and single-ascus isolates of germinated material were obtained on Leonian's agar. Cultures were allowed to remain at room temperature for 21 days before being placed at the low temperature. Rate of growth at either temperature was extremely slow. Within 21 days at room temperature colonies of heavy, matted, pinkish-white mycelium about 2 cm. in diameter and with aerial portions of white, cottony mycelium were produced. Additional growth at the low temperature also was heavy, matted, and pinkish-white and was produced very slowly. Conidia were not observed in any culture within a period of 14 months.

Two to four mature perithecia were produced in each of three cultures within a period of nine months. Perithecial primordia were not produced in abundance in any culture.

Leptosphaeria heterospora (de Not.) Niessl. (Collection No. 43; FIG. 10)

Isolates of single germinated spores became established on Leonian's agar. Subcultures on this medium and on malt agar were left at room temperature 7–14 days before removal to the cold room. Heavy, gray-green to greenish-black colonies with a copious, gray-green to dark olive, cottony aerial mycelium were produced on Leonian's agar and on malt agar. Conidia were formed abundantly in all colonies. Concentric zonation was not apparent in these cultures when viewed from above, but such zonation was distinct in the subsurface mycelium. Perithecial primordia were not found in any culture of this species within a period of 12 months.

B. *Discussion of Culture Data.*

Certain generalizations can be drawn from comparisons of the cultural behaviors just described.

1. In general, species and some strains of species can be distinguished by their cultural characters. In some cases such characters are obvious, as with *Pleospora rainierensis*, which produces a pinkish-white mycelium, and *C. permunda*, which is a very slow grower, forming colonies only a few millimeters in diameter in several months. In other cases, as with *C. Elynae*, *C. diplospora* (No. 33, growth Type II), *Pleospora infectoria*, and *Leptosphaeria heterospora*, all of which produce an abundant dark mycelium and similar conidia, these differences are not so pronounced. *P. infectoria*, however, produces a non-aerial, non-conidial type of growth on Leonian's agar, differing in this respect from the other three contrasted species, which in turn can be differentiated on the basis of minor variations in the zonation, color, and type of aerial growth of their colonies. Species or strains which produce perithecia and ascospores can, of course, be distinguished by the diagnostic characters of this stage.

2. Different single-spore isolates of the same species (*C. diplospora*) or even of the same collection (*P. trichostoma*) may show variations in morphology, but this is not always true.

3. Within collections of a single species or even within the spores of a single collection (e.g., *C. diplospora*, No. 33) there appear to be strains which are or are not able to produce either conidia or mature perithecia. Whereas there are strains which are unable to produce either conidia or ascospores under the cultural conditions used, there are no strains which produce both conidia and ascospores.

4. With some species there appears to be a difference between single-spore and mass-spore transfers in their ability to survive under the conditions imposed upon them.

STUDIES OF CONIDIAL STAGES

Single ascospores of each of four of the species studied produced growths with conidial stages in pure culture. All of these species (*Pleospora infectoria*, No. 21; *Clathrospora diplospora*, No. 33; *C. Elynae*, No. 34; and *Leptosphaeria heterospora*, No. 43) yielded stages referable to the form-genus *Alternaria*. Because the colonial and conidial morphology of isolates of *C. diplospora*, *C. Elynae*, and *L. heterospora* was so similar, an experiment was devised to determine whether growth under different conditions would result in significant variation in conidial size and thus furnish a means of distinguishing the three species by their conidial stages.

Data were obtained from two different types of experiments: (I) cultures of the three isolates were established on plant stems and observations were recorded on characters usually included in form-species descriptions; (II) cultures were established on four different media and data sufficient for statistical calculations were obtained on conidial morphology.

I. Study of Cultures on Plant Stems

A. *Materials and Methods.*

The origins of the individual conidial strains have been noted in connection with the discussions of their perithecial stages (Culture Studies: General Data). In brief, each *Alternaria* strain was

derived from a single ascospore of its perfect stage. For the purpose of this study one conidial strain was selected at random from those available for each perfect stage.

In this initial attempt at establishing the form-species identities of the isolates, cultures were initiated on dead, sterile stems of *Chrysanthemum* sp. which were moistened with distilled water. Such a substratum was used for the purpose of approximating conditions which might be found in nature.

Conidia for starting subcultures were obtained from the original isolates on Leonian's agar. Inoculations on new substrata were made from suspensions of conidia in sterile water. Using a wire loop, a single drop of such a suspension was introduced into each test-tube containing stems. All cultures were maintained at room temperature in diffuse light during daylight hours. Two identical series were maintained.

Cultures of the three different *Alternaria* isolates were examined on the tenth day after inoculation and descriptions of the material observed were written. These descriptions follow in outline the procedure in general use in the literature descriptive of such fungi.

FIGS. 11–13. Conidia and conidiophores derived in culture from isolated ascospores. 11. *Clathrospora diplospora* (E. & E.) Wehm. in press (collection No. 33). 12. *Clathrospora Elynae* Rab. 13. *Leptosphaeria heterospora* (de Not.) Niessl.

B. *Experimental Results: Descriptions of Conidial Stages.*

Clathrospora diplospora (E. & E.) Wehm. In press (conidial stage; FIG. 11)

Aerial mycelium loose cottony to compact cottony, abundant, light gray to gray-green to olive. *Hyphae* hyaline to olive to dark

olive-brown, septate, branching, usually 3.6–4.8 μ wide. *Conidiophores* medium to dark olive, septate, mostly 21.8–66.6 × 3.6–4.9 μ but often bearing conidia when no longer than 2.4 μ, usually erect, simple and straight with a single scar at apex or, rarely, branched and geniculate with a scar at the apex of each branch and at each bend, swollen slightly at apex, borne separately on aerial hyphae, appearing separately on surface of plant substratum or frequently clustered in groups of two or more. *Conidia* smooth or verrucose in the same chain or even on the same spore (upper portion smooth, lower portion verrucose), surface topography not definitely correlated with relative age (i.e., with position of individual spore in the chain); in shape [2] beakless oval, short or long oval, short or long cone, or cylindrical; 10.9–55.7 × 6.1–14.5 μ; hyaline to pale olive to medium olive to olive-brown; septation 1–8 transverse and 0–6 longitudinal; beak present or, rarely, absent, transition from body of spore to beak gradual or abrupt, beak appearing as a scarcely differentiated apical cell (but usually lighter in color than cells of the spore body) or as a distinctly elongated, simple or branched, straight or geniculate rostrum, 2.4–6.0(–21.8) × 2.4–4.8 μ, concolorous with or slightly lighter than the body cells; in chains of 5–10(–20); chains simple or branched.

Clathrospora Elynae Rab. (Conidial stage; FIG. 12)

Aerial mycelium loose cottony, light gray to dark olive. *Hyphae* hyaline to medium olive, septate, seldom deviating from 3.6 μ in width. *Conidiophores* olive-brown, septate, mostly 36.2–55.7 × 4.2 μ but often bearing conidia when as short as 2.4 μ, erect to inclined, not seen to branch or to become geniculate, with terminal scar, slightly swollen at apex, decidedly swollen at base, usually solitary on hyphae but occasionally appearing as paired or bunched on surface of substratum. *Conidia* usually verrucose, but smooth when young; in shape beakless oval, short or long oval, short or long cone, or, rarely, cylindrical; 9.7–36.3 × 6.1–12.1 μ; hyaline to medium olive to dark olive-brown; septation 1–6 transverse and 0–4 longitudinal; beak (when present) simple or, rarely, geniculate with a scar at apex and at each bend, short (appearing scarcely differentiated from body cells except by lighter color) or elongate, 1.2–18.2 × 2.4–3.6 μ; in chains of 5–10 or frequently more; chains usually simple but occasionally branching.

[2] Descriptive terms applied to shapes of conidia are based on those defined by Neergaard (**1945**).

Leptosphaeria heterospora (de Not.) Niessl. (Conidial stage; FIG. 13)

Aerial mycelium loose cottony, light to dark gray. *Hyphae* hyaline to light green to dark olive, septate, branching, 2.4–3.6 μ wide. *Conidiophores* medium olive, septate, up to 54.5 × 4.8 μ in size, erect or inclined, not seen to branch but some geniculate, scars at apex and at bends, not noticeably swollen at apex, borne singly on hyphae but appearing clustered on substratum. *Conidia* smooth or verrucose; in shape short or long oval, short or long cone, or cylindrical; 10.9–31.5 × 6.1–10.9 μ; hyaline to medium olive to dark olive; septation 1–6 transverse and 0–3 longitudinal; beak present as scarcely differentiated apical cell or as elongate, septate rostrum, simple, sometimes geniculate, with scar at apex and at each bend, tapering from body of spore to rostrum or with abrupt transition, 1.2–20.6 × 2.4–4.2 μ; in chains of 3–10 or more; chains simple or branched.

C. *Discussion.*

Identification of these individual isolates of *Alternaria* on the basis of the descriptions given and with the use of the modern taxonomic treatment of the form-genus by Neergaard (**1945**) necessitates their placement under the binomial *Alternaria tenuis auct. sensu str. Clathrospora diplospora, C. Elynae,* and *Leptosphaeria heterospora* cannot be distinguished in their conidial stages by the use of ordinary morphologic criteria. Although it is evident that the isolates of these species are genetically distinct, they must be regarded as members of the form-species *Alternaria tenuis.* It is apparent from this experiment that form-species as described in the literature may represent the imperfect stages of a number of species, either in one genus or distributed in closely related genera. It follows, then, that the perfect-stage binomial cannot logically be applied to a single form-species on the basis of a single connection of a perfect and a conidial stage.

II. Study of Cultures on Artificial Media with a Statistical Analysis of Conidial Length

A. *Materials and Methods.*

Each *Alternaria* strain was derived from a single ascospore of its perfect stage, as indicated previously.

Cultures used as the sources of data for statistical studies were carried on four different media, each medium being used at an acid pH and at an alkaline pH. These media were Leonian's agar (pH 4.9 and pH 7.7), oatmeal agar (pH 5.7 and pH 7.7), mineral-peptone agar (pH 5.1 and pH 7.7), and mineral agar (pH 5.2 and pH 7.7). It should be noted that three of the media (Leonian's, mineral-peptone, and mineral agars) comprise a series which is decreasingly complex in composition. Such a series of substrata was used in an attempt to discover and to define, as nearly as possible, culture conditions under which the different isolates could be distinguished or under which variation within single isolates would occur.

Conidia for starting subcultures were obtained from the original isolates on Leonian's agar. Inoculations on new media in each series were made from suspensions of conidia in sterile distilled water. Using a wire loop, a single drop of such a suspension was placed in the center of each Petri dish of artificial medium. All cultures were maintained under the same conditions—room temperature and diffuse light during daylight hours. Two identical series were maintained.

Previous observations indicated that under the best conditions for growth some of the colonies would extend to the side-walls of the Petri dishes within a growth-period of ten days. For this reason nine days was fixed as the maximum period within which data could be taken from cultures before mechanical interference with radial growth occurred. In order to have comparative data referable to colony age, the nine-day period was divided arbitrarily into three successive intervals of three days each, data being obtained from each culture at the ends of the three-, six-, and nine-day periods.

The sampling method consisted of dragging a sterile wire loop across the surface of the mycelium in a radius of a colony. (Succeeding samples from a colony were not taken from the same radius.) The spores obtained were mounted in Amman's mounting fluid. Using a mechanical stage, the mount was moved laterally across the microscope field. Data were recorded on all conidia encountered up to a total of 50 spores per mount (except in the few cases when this number of spores could not be found in a

single mount). The data consisted of measurements in ocular micrometer divisions (to the nearest division) of length, excluding the beak, and of width of the spores. These measurements were transposed into micron values for reference purposes and the length ranges in microns are listed in subsequent tables. However, all calculations are based on the original data expressed in ocular micrometer divisions.

B. *Experimental Results.*

Preliminary calculations on width data revealed that the degree of variation of this character within individual species and between different species was so small that neither intraspecific nor interspecific differences could be established. Calculations on length-width ratios were not sufficiently different from similar calculations on length data alone to warrant their use. For these reasons statistical calculations were carried out only on spore-length data, particularly since this character offered a decidedly greater range of values.

Making use of standard statistical formulae, the following values were determined from the length data of each sample: range of length, mean length, and standard deviation of length (TABLES II and III). The mean length values and the standard deviation of length values then were used in comparing data obtained from different samples by employing them in a standard ratio designed as a test for significance of difference between means (TABLES IV–VII).

C. *Discussion of Tables.*

TABLES II and III summarize the actual length ranges plus derived values for samples of the three isolates on the four media used, at three different ages, and at the acid (TABLE II) and the alkaline (TABLE III) reactions used. In these tables the spore-length ranges of the different samples show a great degree of overlapping between and within individual isolates. No general statement can be made on species variation or differentiation on the basis of these length extremes alone. Similarly, the mean length and standard deviation of length values do not fall into a pattern

TABLE II

RANGE OF LENGTH, MEAN LENGTH, AND STANDARD DEVIATION OF LENGTH OF CONIDIA SAMPLES FROM *Alternaria* CULTURES OF DIFFERENT SPECIES ORIGIN, OF DIFFERENT AGES, AND PRODUCED ON DIFFERENT MEDIA WITH pH IN THE ACID RANGE

Sample	R	Rμ	m	σ
33–L–3–a	10–41	11.70–47.97	25.04	6.64
–6–	9–27	10.53–31.59	17.78	4.86
–9–	8–35	9.36–40.95	21.74	5.39
33–O–3–a	5–43	5.85–50.31	22.30	8.55
–6–	10–40	11.70–46.80	22.58	7.36
–9–	11–30	12.87–35.10	19.70	4.85
33–P–3–a	9–38	10.53–44.46	18.56	7.02
–6–	10–30	11.70–35.10	16.84	4.24
–9–	10–27	11.70–31.59	16.34	3.21
33–M–3–a	7–41	8.19–47.97	17.08	6.73
–6–	6–28	7.02–32.76	15.32	5.62
–9–	10–30	11.70–35.10	16.12	3.85
34–L–3–a	8–32	9.36–37.44	19.64	6.37
–6–	10–35	11.70–40.95	18.76	5.87
–9–	8–25	9.36–29.25	15.12	3.52
34–O–3–a	13–30	15.21–35.10	20.56	4.88
–6–	8–23	9.36–26.91	15.20	3.75
–9–	8–25	9.36–29.25	15.04	3.85
34–P–3–a	——	——	——	——
–6–	——	——	——	——
–9–	10–20	11.70–23.40	14.16	2.36
34–M–3–a	——	——	——	——
–6–	——	——	——	——
–9–	8–18	9.36–21.06	11.72	2.47
43–L–3–a	8–37	9.36–43.29	21.96	6.11
–6–	9–37	10.53–43.29	20.68	6.31
–9–	11–38	12.87–44.46	21.14	5.91
43–O–3–a	9–35	10.53–40.95	19.48	5.80
–6–	9–31	10.53–36.27	18.30	5.32
–9–	11–26	12.87–30.42	18.50	4.00
43–P–3–a	10–25	11.70–29.25	17.26	3.91
–6–	9–26	10.53–30.42	16.00	4.10
–9–	10–26	11.70–30.42	15.54	3.85
43–M–3–a	7–28	8.19–32.76	15.90	5.28
–6–	8–22	9.36–25.74	15.14	3.02
–9–	9–25	10.53–29.25	14.38	4.40

definite enough for the formulation of any unqualified conclusion. The most obvious generalities to be noted in these tables are: (1) the mean length and the standard deviation of length are, in most cases, greatest at the end of the first three-day growth period, and (2) mean lengths and standard deviations of lengths show a general pattern of decrease in value in samples from the more complex to the less complex media.

TABLE III

RANGE OF LENGTH, MEAN LENGTH, AND STANDARD DEVIATION OF LENGTH OF CONIDIA SAMPLES FROM *Alternaria* CULTURES OF DIFFERENT SPECIES ORIGIN, OF DIFFERENT AGES, AND PRODUCED ON DIFFERENT MEDIA WITH pH IN THE ALKALINE RANGE

Sample	R	Rμ	m	σ
33–L–3–b	11–36	12.87–42.12	22.60	5.07
–6–	10–40	11.70–46.80	19.86	6.32
–9–	11–50	12.87–58.50	22.68	7.66
33–O–3–b	11–36	12.87–42.12	20.36	5.56
–6–	11–29	12.87–33.93	20.26	4.35
–9–	11–32	12.87–37.44	18.40	4.61
33–P–3–b	12–30	14.04–35.10	17.80	5.06
–6–	10–33	11.70–38.61	19.32	5.45
–9–	11–37	12.87–43.29	19.84	7.20
33–M–3–b	9–32	10.53–37.44	18.16	4.29
–6–	10–28	11.70–32.76	16.78	4.10
–9–	9–29	10.53–33.93	17.40	4.47
34–L–3–b	6–33	7.02–38.61	18.14	5.20
–6–	7–23	8.19–26.91	14.96	3.67
–9–	10–29	11.70–33.93	16.08	3.68
34–O–3–b	8–27	9.36–31.59	15.74	4.48
–6–	10–23	11.70–26.91	15.54	3.08
–9–	10–26	11.70–30.42	15.24	3.52
34–P–3–b	7–28	8.19–32.76	14.96	4.49
–6–	9–28	10.53–32.76	13.88	4.16
–9–	9–28	10.53–32.76	13.46	3.94
34–M–3–b	7–26	8.19–30.42	15.28	4.35
–6–	9–26	10.53–30.42	15.74	4.18
–9–	8–20	9.36–23.40	12.32	2.94
43–L–3–b	7–36	8.19–42.12	21.72	6.18
–6–	10–29	11.70–33.93	19.68	5.34
–9–	9–35	10.53–40.95	18.94	5.48
43–O–3–b	8–30	9.36–35.10	18.54	5.10
–6–	10–26	11.70–30.42	17.50	4.33
–9–	10–28	11.70–32.76	16.98	4.40
43–P–3–b	11–28	12.87–32.76	17.00	3.88
–6–	8–30	9.36–35.10	16.52	4.42
–9–	9–27	10.53–31.59	16.58	4.46
43–M–3–b	10–30	11.70–35.10	15.72	3.91
–6–	8–22	9.36–25.74	13.44	3.94
–9–	9–28	10.53–32.76	15.44	3.89

In TABLES II–VII the following symbols have been used in order to simplify and to standardize the method of reference to the individual samples and to the comparison of two different samples: 33, 34, 43 (cultures derived respectively from *Clathrospora diplospora*, *C. Elynae*, and *Leptosphaeria heterospora*); 3, 6, 9 (ages of cultures in days); L, O, P, M (substrata respectively Leonian's agar, oatmeal agar, mineral-peptone agar, and mineral agar); a, b (pH's of media respectively in acid or in alkaline range); R (range of length in ocular micrometer divisions); Rμ (range of length in microns); m (mean length in ocular micrometer divisions); σ (standard deviation of length). In TABLES IV–VII values of *2.0* or more indicate that there exists a significant difference between mean length values under the conditions noted.

TABLES IV, V, and VI list values from which observations may be drawn on the effect upon individual isolates of differences in media, in pH, and in age of culture.

Individual isolates of a species show striking differences when mean lengths of conidia from different media are compared (TABLE IV). In this respect *Leptosphaeria heterospora* is the most notable of the three species studied. Here significant differences in conidial lengths are present between samples from all pairs of media in the acid range excepting the mineral-peptone–mineral-agar

TABLE IV

DEGREE OF SIGNIFICANCE OF THE DIFFERENCE BETWEEN MEAN LENGTHS OF CONIDIA SAMPLES FROM CULTURES OF THE SAME AGES AND SIMILAR pH, BUT PRODUCED ON DIFFERENT MEDIA

	Clathrospora diplospora			*Clathrospora Elynae*			*Leptosphaeria heterospora*		
	3	6	9	3	6	9	3	6	9
L–O–a	1.8	*3.8*	*2.0*	0.7	*3.2*	0.1	*2.1*	*2.0*	*2.6*
L–P–a	*4.7*	1.0	*6.1*	—	—	1.4	*4.6*	*4.4*	*5.6*
L–M–a	*5.9*	*2.3*	*6.0*	—	—	*4.7*	*5.3*	*5.6*	*6.5*
O–P–a	*2.4*	*4.8*	*4.1*	—	—	1.2	*2.2*	*2.4*	*3.7*
O–M–a	*3.4*	*5.5*	*4.1*	—	—	*4.5*	*3.2*	*3.6*	*4.9*
P–M–a	1.1	1.5	0.3	—	—	*3.5*	1.5	1.2	1.4
L–O–b	*2.1*	0.4	*3.4*	*2.5*	0.9	1.2	*2.8*	*2.2*	*2.0*
L–P–b	*4.8*	0.5	1.9	*3.3*	1.4	*3.4*	*4.6*	*3.2*	*2.4*
L–M–b	*4.7*	*2.9*	*4.2*	*3.0*	1.0	*5.6*	*5.8*	*6.6*	*3.7*
O–P–b	*2.4*	1.0	1.2	0.9	*2.3*	*2.4*	1.7	1.1	0.5
O–M–b	*2.2*	*4.1*	1.1	0.5	0.3	*4.5*	*3.1*	*4.9*	1.9
P–M–b	0.4	*2.6*	*2.0*	0.4	*2.2*	1.6	1.6	*3.7*	1.4

series, i.e., in 83% of compared pairs. Similar differences are noted in the alkaline pH range, in which 67% of pairs of media compared yield samples significantly different. Since all media in the acid range do not have the same pH, it might be argued that the definite differences in mean lengths are due to the reaction differences of the media. This factor, however, appears to have little effect on mean lengths, as can be judged from specific pH comparisons in TABLE V. The comparisons in TABLE V cover a much greater range of pH value than is found between any two different media of the acid range. Consequently, it can be concluded that the large number of significant differences in mean length noted in TABLE IV, particularly for *Clathrospora diplospora* and *L. hetero-*

spora, reflects the nutritive differences of the media rather than the pH differences. In this connection it should be noted that in at least two-thirds of the media comparisons for *C. diplospora* and for *L. heterospora* and in as many as one-half of similar compari-

TABLE V

DEGREE OF SIGNIFICANCE OF THE DIFFERENCE BETWEEN MEAN LENGTHS OF CONIDIA SAMPLES FROM CULTURES OF THE SAME AGES PRODUCED ON MEDIA DIFFERING ONLY IN pH

	Clathrospora diplospora			*Clathrospora Elynae*			*Leptosphaeria heterospora*		
	3	6	9	3	6	9	3	6	9
L–a–b	*2.1*	1.8	0.7	1.3	*3.9*	1.3	0.2	0.9	1.9
O–a–b	1.3	1.9	1.4	*4.1*	0.4	0.3	0.9	0.8	1.8
P–a–b	0.6	*2.5*	*3.1*	—	—	1.0	0.3	0.6	1.3
M–a–b	1.0	1.5	1.5	—	—	0.9	0.2	*2.4*	1.3

sons for *C. Elynae*, significant differences in mean conidium length are found. The chemical constitution of the substratum clearly has a decided effect upon the length of conidia of these isolates under specified conditions.

For any one isolate there are relatively few instances in which contrasting pH's influenced the production of significantly different mean-length values for samples (TABLE V). The conclusion that the pH of the medium within the ranges used does not have a

TABLE VI

DEGREE OF SIGNIFICANCE OF THE DIFFERENCE BETWEEN MEAN LENGTHS OF CONIDIA SAMPLES FROM CULTURES PRODUCED ON THE SAME MEDIA BUT OF DIFFERENT AGES

	Leonian		Oatmeal		Mineral peptone		Mineral	
	a	b	a	b	a	b	a	b
33–3–6	*6.3*	*2.4*	0.2	0.1	1.5	1.5	1.4	1.6
–3–9	*2.7*	0.1	1.9	1.9	*2.0*	1.6	0.9	0.9
–6–9	*3.8*	*2.0*	*2.3*	*2.1*	0.7	0.4	0.8	0.7
34–3–6	0.7	*3.5*	*4.3*	0.3	—	1.2	—	0.5
–3–9	*4.4*	*2.3*	*4.9*	0.6	—	1.8	—	*4.0*
–6–9	*11.9*	1.5	0.2	0.5	—	0.5	—	*4.7*
43–3–6	1.0	1.8	1.1	1.1	1.6	0.6	0.8	*2.9*
–3–9	0.7	*2.4*	1.0	1.6	*2.2*	0.5	1.6	0.4
–6–9	0.4	0.7	0.2	0.6	0.6	0.1	1.0	*2.6*

decided differential effect upon mean conidial length is borne out by the listed results of pH comparisons.

For the most part, age differences in colonies do not result in significant differences in conidial mean lengths, insofar as the three-, six-, nine-day sampling series can reveal such differences (TABLE VI). An exception to this generality is found in comparisons made with samples from Leonian's agar. Colonies of *C. diplospora* and of *C. Elynae* grown on this medium yielded samples which, in at least two-thirds of the comparable pairs of ages, exhibited significant differences in mean spore length. Such differences are found in a few comparisons for other media, but, as stated, significant differences in mean length usually are not correlated with age differences.

The most important over-all conclusion to be drawn from the data presented in the different tables is that under certain conditions measurements of conidia can be used to differentiate pure strains of the species studied. Evidence for or against the use of statistical data for differentiating these isolates may be indicated as follows:

1. If the isolates can be separated by these means, then, in a statistical comparison of conidia of two isolates grown in a parallel manner under a number of different conditions, significant mean-value differences should be found under at least some of these conditions.

For the isolates of species used these differences are evident from data in TABLE VII. In comparing *C. diplospora* and *C. Elynae* it is seen that significant differences exist between samples in 17 of the 20 possible conditions of comparison. In a similar comparison of *C. Elynae* and *L. heterospora* it is observed that significant differences exist in 16 of 20 conditions of comparison. With *C. diplospora* and *L. heterospora* significant differences exist only in 11 of the 24 possible conditions of comparison observed.

It follows from these observations that the *Alternaria* isolates of *C. Elynae* can be differentiated statistically from the *Alternaria* isolates of both *C. diplospora* and *L. heterospora* under most of the conditions cited. This is considered significant for species recognition in this group of isolates. It also is evident from these observations that the conidial stages of *C. diplospora* and *L. hetero-*

spora can be differentiated by statistical correlations of this type but under a smaller number of conditions. Although the latter two species are separable under only about half of the growth conditions imposed, species recognition by statistical methods definitely is possible insofar as the isolates used are concerned.

TABLE VII

DEGREE OF SIGNIFICANCE OF THE DIFFERENCE BETWEEN MEAN LENGTHS OF CONIDIA SAMPLES OF DIFFERENT SPECIES FROM CULTURES OF THE SAMES AGES ON THE SAME MEDIA

	Leonian		Oatmeal		Mineral peptone		Mineral	
	a	b	a	b	a	b	a	b
33–34–3	*4.2*	*4.3*	1.1	*4.6*	—	*3.0*	—	*3.3*
–6	0.9	*4.7*	*5.7*	*6.3*	—	*5.6*	—	1.3
–9	*7.3*	*5.5*	*5.2*	*3.8*	*3.3*	*5.5*	*5.9*	*6.7*
34–43–3	*2.3*	*3.1*	0.8	*2.9*	—	*2.4*	—	0.5
–6	1.9	*5.0*	*2.9*	*2.6*	—	*3.1*	—	*2.8*
–9	*6.0*	*3.1*	*4.4*	*2.2*	1.9	*3.7*	*3.3*	*4.5*
33–43–3	*3.1*	0.8	1.9	1.7	1.1	0.9	1.0	*3.0*
–6	*2.9*	0.2	*3.3*	*3.2*	1.0	*2.8*	0.2	*4.2*
–9	0.6	*2.8*	1.3	1.6	1.1	*2.7*	*2.1*	*2.3*

2. If isolates of these species can be differentiated statistically, certain pairs of conditions should exist between which mean lengths of conidia of one and the same species do not show significant differences, while at the same time mean lengths of conidia of two different species under the same conditions do show significant differences.

Such comparisons may be made using data from TABLES IV, V, and VI in conjunction with TABLE VII. In comparing culture pairs of isolates of two species such as *C. Elynae* and *C. diplospora* comparable to the culture pairs of *C. Elynae* alone which do not show a significant difference in conidial mean length, it is found that differentiation of isolates can be established in at least 82% of the comparisons. In all such comparisons of cultures of *C. Elynae* with those of *C. diplospora* and *L. heterospora*, species identity of the isolates can be established at least two-thirds of the time. However, in comparisons of *C. diplospora* and *L. heterospora* species differentiation of the isolates, though possible, can

be established in not more than 21% of the cases. These observations are summarized in TABLE VIII.

In summary, it is possible, in the absence of data sufficient for identification of species in ordinary descriptive material of these conidial stages, that statistical data based on measurements of conidia from isolates in pure culture may be of value in the recognition of species. Specifically, by the use of the culture methods

TABLE VIII

COMPARISON OF NUMBER OF PAIRS OF CONDITIONS UNDER WHICH ONE ISOLATE DOES NOT SHOW SIGNIFICANT DIFFERENCES WITH NUMBER OF COMPARABLE CONDITIONS UNDER WHICH TWO ISOLATES DO SHOW SIGNIFICANT DIFFERENCES

No. of pairs of conditions for one isolate which *do not* yield significant differences		No. of comparable conditions for two isolates which *do* yield significant differences		% of comparisons in which differentiation between isolates can be established
Isolate	No.	Isolates	No.	%
33	27	33–34	20	74.1
34	28	34–33	23	82.1
34	28	34–43	20	71.4
43	30	43–34	20	66.7
33	38	33–43	8	21.1
43	40	43–33	7	17.5

and of the statistical methods indicated, the *Alternaria* stage of the isolate of *Clathrospora Elynae* can be differentiated from the similar isolates of *C. diplospora* and *Leptosphaeria heterospora* under a large percentage of culture conditions. However, such differentiation is possible in a relatively small percentage of comparisons involving conidial stages of isolates of *C. diplospora* and *L. heterospora* only.

GENERAL DISCUSSION

Since variation in spore length has been used extensively in the definition of species of *Alternaria*, particular attention has been paid in this study to the constancy of the length ranges of spores of certain species. The view maintained here should be contrasted with that in recent taxonomic investigations on this group of fungi.

The results of previous investigations on conidial morphology of certain fungi have been used, in conjunction with culture char-

acters, as bases for extending the descriptions of species to include increasingly diverse forms. This interpretation has resulted in the necessity for reducing numbers of binomials to synonymy in the Fungi Imperfecti.

In one recent instance the circumscription of *Alternaria tenuis auct.* is such that specimens exhibiting similar spore and conidiophore morphology are included, although isolates from these collections exhibit quite distinct differences when cultural characters are compared (Neergaard, **1945**). (To be noted particularly in this connection are Neergaard's statements concerning the appearance of colonies in pure culture on artificial media and also the plant substratum specificity or preferences of the individual isolates.) Here the implication is that we are concerned with a number of naturally related forms differing in slight detail.

Groves and Skolko (**1944**), who also studied numerous isolates of *Alternaria tenuis,* recognized different patterns of variation in spore size in different strains of this form-species. On the basis of their observations on several variable spore characters, however, they concluded that "the only workable method of handling these forms was to group them all together and consider them as strains or morphotypes of one species." Their concept resulted in the synthesis with *A. tenuis* "of a great many *Alternaria* species including *A. fasciculata* (E. & M.) Jones & Grout, *A. humicola* Oud., *A. maritima* Sutherland, and many other species that have been described chiefly on the basis of the host."

In other recent work on synonymy of form-species Snyder and Hansen (**1940**, **1941**, and **1945**), in their taxonomic treatment of sections of the form-genus *Fusarium,* have grouped under 10 binomials the multitude of Fusaria previously described. In addition, these authors have placed under eight binomials at least 22 described ascomycetous forms reported to be ontogenetically related to the Fusaria. Their taxonomic treatment of the perithecial stages apparently was based not on the systematic merits of the perfect stages themselves but primarily on the basis of the form-species delimitations of their conidial counterparts. The taxonomic changes suggested by these authors are based to a great degree on data from culture work and are intended to increase the usability

of the *Fusarium* taxonomy. However, in no case do they arrive at the definition of a natural species in the group.

Such procedures as these in taxonomic work must be understood for what they are, namely, methods by which populations circumscribed on the basis of morphologic characters may be assigned binomials. The distinction between the concepts of the form-genus and of the form-species, as applied to the Fungi Imperfecti, and the concepts of the genus and of the species, as usually applied to perfect stages, must be carefully maintained.

In none of the studies reviewed above and in no other definitive work encountered on species of these form-genera has the conidial material for comparisons been derived directly from known ascomycetous stages. The point is granted that it is not practical and often, in fact, it may not be possible to connect perfect stages with known conidial stages. This is borne out well by the fact that many distinct form-species of *Alternaria* are described for which no perfect stages are known. However, it is not granted that previous taxonomic work on this and similar form-genera presents more than a more or less usable means of identifying form-species encountered. In no known instance in the literature are the individual isolates considered as separate genetic entities if they correspond well with an existing description based on morphology. In fact, the close correspondence of an isolate with an established description usually is accepted as a means of strengthening the concept of the form-species described, which in turn is supposed to represent the asexual counterpart of the perfect or sexual stage of a single species.

If the definition of a given form-species becomes so concrete that it is accepted in the sense of a species instead of merely a portion of a life cycle, objections must be raised. This increasingly common practice of treating morphologically similar populations of the Fungi Imperfecti as genetically similar or identical species is based on false premises, in that such similar populations need not necessarily have any great degree of genetic similarity. This point has been illustrated here by the work presented on three different isolates of the form-species *Alternaria tenuis*, each isolate

having been derived from an ascospore of a different perfect stage. In this instance the over-all morphology of many subcultures and of the spores of the three different species remained within the modern descriptive limits of *A. tenuis*, but the fact of the diverse origins of the isolates upholds the view that this binomial must be considered as a name given for the imperfect stages of many morphologically similar but genetically different organisms.

The statistical work presented here on the *Alternaria* stages of *Clathrospora diplospora*, *C. Elynae*, and *Leptosphaeria heterospora* is intended to be descriptive of these fungi rather than diagnostic. These three strains of the imperfect stages can, in fact, be distinguished from each other by statistical methods when grown under certain environmental conditions. The cultural and statistical techniques outlined could be applied to differentiation of individual strains of the same or of different conidial stages. It is not illogical to expect, however, that equally as definite statistical differences would be obtained between conidial samples from different ascospore isolates of one and the same species under some environmental conditions. The total conidial variability of one species, as reflected by variations observed in numerous isolates of the organism, would not necessarily be distinct from the total conidial variability of another species. In consequence, studies of the total variation of conidial stages are not expected to be primarily diagnostic in value.

For critical work on specific imperfect stages it should be recognized that the degree of variation between isolates of the same genetic species may be no greater or even less than that between isolates of different species or even genera, as recognized upon the characters of the perfect stage. If this fact is kept in mind, revisions in descriptive technique may be guided by statistical methods, as presented herein, or by well-established cultural procedures already used in bacteriology and mycology, such minor differences being recognized, however, as indicative of strain, specific, or generic rank only after careful correlation with the proper perfect or sexual stage. As has been stated, the statistical work on this form-species of *Alternaria* is intended to be descriptive of the variability of the genetically distinct isolates. It is not anticipated

that any taxonomic changes will be derived from such limited observations. On the other hand, it is asserted with conviction that the form-species of the Fungi Imperfecti (in particular *Alternaria tenuis*) must be treated in both theoretical and applied fields of mycology as synthetic groupings until individual exceptions can be proved. It is suggested that variation established in cultures of an organism derived from a known source may be used as a basis for interpreting the variation in populations found occurring naturally.

SUMMARY

1. Isolations were attempted with ascospores from perithecia of 43 collections of species of *Pleospora, Clathrospora,* and *Leptosphaeria.*

a. Three of 12 isolates which became established in culture (in addition to cultures of *Pleospora infectoria* received from another source) produced conidial stages in culture. These were *Clathrospora diplospora, C. Elynae,* and *Leptosphaeria heterospora.*

b. Six other isolates of the 12 established in culture produced mature perithecia. These were *Pleospora ambigua, P. njegusensis, P. trichostoma, Clathrospora diplospora,* an isolate of a species identified as a "large-spored variety" of *C. diplospora,* and *P. rainierensis.*

c. In no case was it observed that a conidial strain also produced perithecia or that a perithecial strain also produced a conidial stage in culture.

2. The characters of the different isolates in culture were sufficiently distinct to permit the recognition of the individual species insofar as the isolates used were concerned.

3. Identification of the conidial stages of *Clathrospora diplospora,* of *C. Elynae,* and of *Leptosphaeria heterospora* by means of commonly accepted descriptions necessitated their placement in the form-species *Alternaria tenuis.*

4. Statistical analyses of conidial lengths observed in strains of *Clathrospora diplospora,* of *C. Elynae,* and of *Leptosphaeria heterospora* indicated that, under certain environmental conditions, the

strains studied of each of the species could be differentiated from each of the other two.

5. Nutrient differences in media upon which the *Alternaria* strains were grown resulted in a greater percentage of statistical differences in comparisons among the strains than did pH or age differences.

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COLLECTIONS USED [3, 4]

Pleospora ambigua (Berl. & Bres.) Wehm.

1. On *Lupinus albicaulis* var. *shastensis*, Mt. Shasta, Calif., 1941, leg. W. B. Cooke No. *15739*.
2. On *Aster shastensis*, Mt. Shasta, Calif., 1946, leg. W. B. Cooke No. *18234*.
3. On *Lupinus subalpinus*, No. *1424*.
4. On *Chrysanthemum leucanthemum*, No. *1812*.
5. On *Artemisia tacomensis*, No. *1966*.
6. On *Valeriana sitchensis*, No. *2338b*.

Pleospora asymmetrica Wehm.

7. On *Lupinus volcanicus*, No. *1937b*.
8. On *Agrostis* sp., No. *2187*.
9. On *Festuca viridula*, No. *2188*.
10. On *Muhlenbergia filiformis*, No. *2200a*.

Pleospora comata Niessl

11. On *Silene Macounii*, No. *1969*.

Pleospora helvetica Niessl

12. On *Lupinus subalpinus*, No. *1613*.
13. On *Aster foliaceus*, No. *1670*.
14. On *Pedicularis contorta*, No. *1673*.
15. On *Arnica Parryi*, No. *1935*.
16. On *Lupinus volcanicus*, No. *1937a*.
17. On *Ligusticum purpureum*, No. *1952b*.
18. On *Achillea millefolium* var. *alpicola*, No. *1958*.

Pleospora herbarum (Pers.) Rab.

19. On *Chrysanthemum leucanthemum*, No. *1812a*.

Pleospora herbarum (Pers.) Rab. var. *occidentalis* Wehm.

20. On *Artemisia tacomensis*, No. *1929*.

[3] All specimens were identified by Doctor L. E. Wehmeyer. The collection of *Pleospora infectoria* (No. 21) was obtained through the courtesy of Marvin D. Whitehead, who states that it is the type specimen of *Pyrenophora alternarina* Whitehead & Dickson. The name given by Whitehead and Dickson is used here in reference, although the specimen is listed as *Pleospora infectoria* Fuckel, from which species Wehmeyer considers the specimen to be morphologically indistinguishable.

[4] All collections not noted otherwise were made by the author in Mt. Rainier National Park, Wash., in the summer of 1948. Collection numbers are noted.

Pleospora infectoria Fckl.

21. On oat straw (*Avena sativa*), Madison, Wisc., 1948, leg. M. D. Whitehead.

Pleospora laxa Ell. & Holw.

22. On *Potentilla flabellifolia*, No. *1951*.

Pleospora njegusensis Bub.

23. On *Helianthella* sp., Pullman, Wash., 1948, leg. W. B. & V. G. Cooke No. *23487*.

24. On *Balsamorrhiza sagittata*, near Moran, Wyo., 1948, leg. E. G. Simmons No. *1370*.

Pleospora oligasca Bub.

25. On dead stems of a rosaceous plant, between Red Lodge, Mont., and N.E. entrance of Yellowstone National Park, Wyo., 1948, leg. E. G. Simmons No. *1366*.

Pleospora richtophensis E. & E. var. *pallida* Wehm. Inedit.

26. On *Ranunculus* sp., Blue Mt. Summit Pass, Ore., 1948, leg. E. G. Simmons No. *1374*.

27. On *Aster foliaceus*, No. *1926*.

Pleospora rubicunda Niessl var. *americana* Wehm. Inedit.

28. On *Potentilla flabellifolia*, No. *1951a*.

Pleospora trichostoma (Fr.) Ces. & de Not.

29. On old wheat straw, Riley Co., Kansas, 1949, leg. T. E. Brooks No. *1945*.

Clathrospora Cookei Wehm. Inedit.

30. On *Stipa californica*, Mt. Shasta, Calif., 1946, leg. W. B. Cooke No. *18095*.

Clathrospora diplospora (E. & E.) Wehm. In press

31. On *Monardella odoratissima*, Mt. Shasta, Calif., 1946, leg. W. B. Cooke No. *18094*.

32. On *Castilleja miniata*, Mt. Shasta, Calif., 1947, leg. W. B. Cooke No. *20284*.

33. On *Anemone occidentalis*, Mt. Shasta, Calif., 1947, leg. W. B. Cooke No. *20285*.

Clathrospora Elynae Rab.

34. On *Juncus mertensianus*, Mt. Shasta, Calif., 1946, leg. W. B. Cooke No. *18427*.

Clathrospora permunda (Cke.) Sacc.

35. On *Ligusticum purpureum*, No. *1952a*.

Pleospora rainierensis Wehm.

36. On stems of a composite, No. *1746d*.

37. On *Muhlenbergia filiformis*, No. *2209*.

Clathrospora Simmonsii Wehm. Inedit.

38. On stems of *Carex ablata*, No. *1930*.

39. On leaves of *Carex ablata*, No. *1931*.

Leptosphaeria Erigerontis Berl.

40. On *Ligusticum purpureum*, No. *1952*.

Leptosphaeria filiformis Wehm. Inedit.

41. On stems of a composite, No. *1746a*.

Leptosphaeria heterospora (de Not.) Niessl

42. On *Trisetum spicatum*, Mt. Shasta, Calif., 1947, leg. W. B. Cooke No. *20223*.

43. On *Poa epilis*, Mt. Shasta, Calif., 1947, leg. W. B. Cooke No. *20477*.

THE HETEROSPORIUM DISEASE OF CALIFORNIA POPPY

LILY H. DAVIS

(WITH 3 FIGURES)

During the early summer of 1947 a disease on California poppy became serious enough to cause reduction in seed yield in commercial plantings of this minor ornamental crop in the Santa Maria Valley. Apparently the disease had been prevalent there for a number of years without causing appreciable loss. The disease has also been seen in the Salinas and Lompoc Valleys.

The fungus, *Heterosporium eschscholtziae* Hark., causing the disease was originally described by Harkness (**6**) in 1884 on living foliage of *Eschscholtzia californica* Cham. in San Francisco, California. There seem to be no subsequent records in the United States of the fungus or the disease until the present one. However, there is a report (**9**) from Copenhagen, Denmark, where it was observed on flowering plants of *Eschscholtzia californica* on October 3, 1941, causing browning of the leaf blades and brown spots on stems and capsules. Dr. P. Neergaard [1] reports that there is little doubt that the fungus is seed-borne but he has not found it on the seed. In Denmark they grow their own seed or import from Holland, but the possibility that the disease came originally from California cannot be excluded.

This paper presents an expanded description of the fungus, details of its life history, and various aspects of the disease it causes.

SYMPTOMS

Although the plant as a whole is attacked, the spots on the mature capsules are the most conspicuous phase of the disease. If the young capsule is infected it may shrivel and turn a faint purplish brown, but when an older capsule is attacked it becomes covered with reddish brown to black elongated spots, 1 mm. to

[1] Letters of July 25 and November 7, 1947.

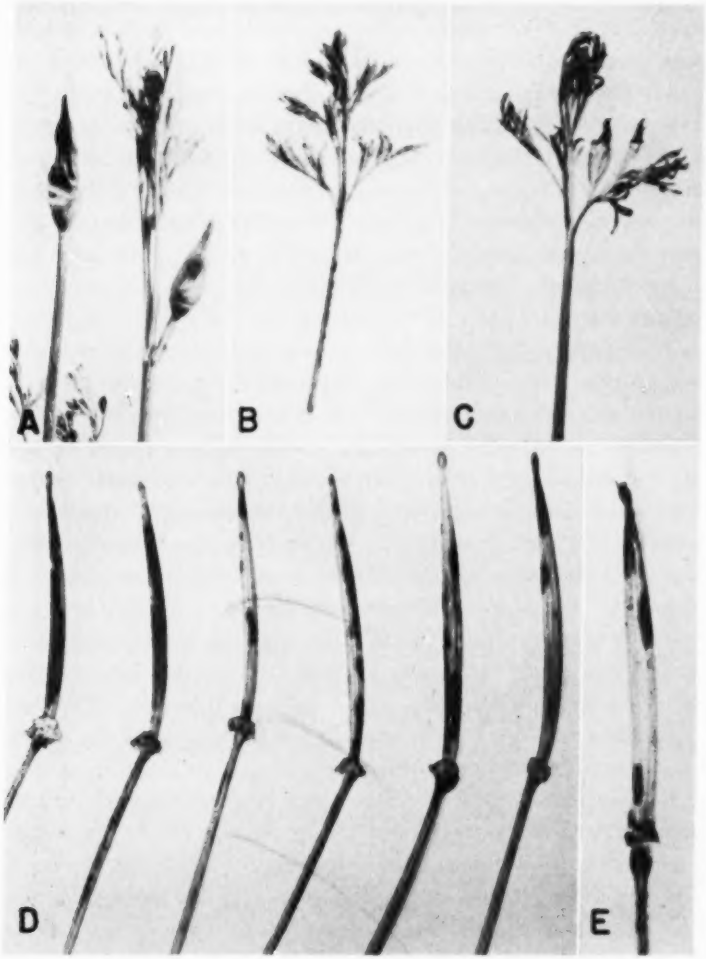

FIG. 1. *Heterosporium* disease of California poppy. A. Infected calyptras from inoculated outdoor material. B. Early stage of leaf infection from inoculated material. C. Later stage of infection showing browned and shrivelled leaf. D. Naturally infected capsules from commercial planting. E. Spots on capsule from inoculated material.

1.5 cm. in size, which eventually coalesce to involve most of the fruit (FIG. 1, D). Under moist conditions these become covered with conidia. No spots were noticed on the flower itself but the fungus frequently attacks the senescent flower parts, receptacle, and peduncle, spreading from these structures to the base of the capsule either preventing the formation of the latter or restricting its growth. In capsules showing no visible symptoms the parasite very often penetrates through the point of attachment and ramifies internally, killing many of the seeds (FIG. 3, A). The apical end is less frequently infected but this sometimes occurs through the senescent styles.

The spots on the stems, petioles, and leaflets start as pin-point red lesions (FIG. 1, B), later coalescing and enlarging to form mature spots of a reddish brown to a deep purplish brown color. In many of the older lesions the centers lighten somewhat and become covered with olive-green spores. Affected leaflets become chlorotic and when the petiole is girdled the whole leaf turns brown, shrivels, and dies (FIG. 1, C). On severely affected plants practically all the leaves wither and die, many becoming covered with spores, but the stems remain green except for the reddish brown to black blotches. In some instances the calyptra is attacked and the young bud turns purple and withers. Plants that have girdling lesions at the crown or main stem usually are chlorotic and stunted.

Seedling infection may be unnoticed in field plantings but is quite pronounced under greenhouse conditions. Much pre- and post-emergence damping-off occurs. Since germination is epigeal the infected seed coats are carried up on the tips of the cotyledons, starting pin-point lesions there and along the hypocotyl, eventually forming brown streaks or purplish areas. Under moist conditions the cotyledons rot and become covered with olive-green spores. In many instances the seedlings wither and die at this stage from girdling lesions at soil level. If conditions are less favorable for the fungus the seedlings may recover by sending out new roots or shoots which generally are chlorotic and stunted. The cankers at the crown are reddish brown and in plants so affected the tap root is generally shriveled.

There are few other diseases of California poppy with which this one might be confused. Powdery mildew, *Erysiphe polygoni* DC.,

produces a somewhat similar spot, but it is generally a deeper purple in color and under slight magnification hyaline mycelium and spores may be seen. This is in contrast to the olive-green spore mass produced by *Heterosporium*. *Entyloma eschscholtziae* Hark. also produces lesions on this poppy in California (**11**) but these spots may be distinguished by the fact that they are small and round rather than elongated and bear characteristic tufts of hyaline basidia. Various Alternarias have been reported (**7**, **8**) as injuring the seed and causing spots on *Papaver* spp. from Europe but not on California poppy. The only other *Heterosporium* found to occur on poppy is *H. groenlandicum* Allescher (**1**) and it occurs only on dead stems of the opium poppy and does not correspond in any way with *H. eschscholtziae*. *Pleospora calvescens* (Fr.) Tul., which produces a serious disease on other Papaveraceae (**4**, **12**), mainly opium poppy, produces symptoms practically identical with those of the Heterosporium disease (**5**, **10**, **13**, **14**). However, it has never been found on *Eschscholtzia californica* and is unknown in North America.

THE PATHOGEN

Heterosporium eschscholtziae was consistently isolated from diseased plants. Although the only known specimen of this fungus was destroyed in the San Francisco fire, there is no doubt that the organism considered here is the same as that described by Harkness (**6**).

The conidiophores (FIG. 2, A, B) are brown, very short, 34–90 × 3.5–5 μ (average 81 × 4.4 μ), under extremely moist conditions becoming even longer; they occur singly or rarely in groups of two or three, differing in this respect from the characteristically fasciculate Heterosporiums but resembling *H. tropaeoli* (**2**, **3**). They vary in septation, are geniculate, with prominent scars, and emerge between or through epidermal cells rather than through stomata.

The yellowish-brown conidia are cylindrical with obtuse ends, one end having a prominent scar. Normally they occur singly but in culture may be borne in sub-chains of two spores. They vary from minutely echinulate to verrucose (older spores) (FIG. 2,

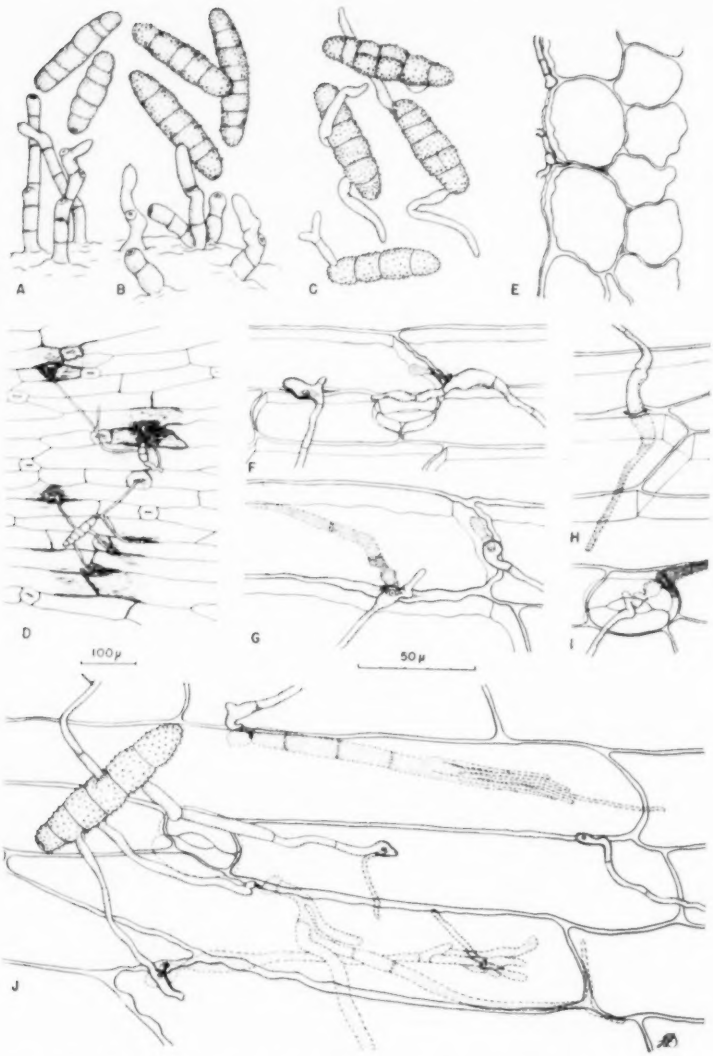

FIG. 2. *Heterosporium eschscholtziae* on poppy. Magnification of A–C, E–J indicated by 50 μ scale, D by 100 μ scale. Stages of penetration, F–J, shown in epidermal strips (gum reaction not included) at various periods of time after inoculation. A. Conidiophores and conidia from moist seedlings. B. Conidiophores and conidia from drier conditions on infected capsule. C. Conidia germinating on water agar after 1½–2½ hours. D. Surface view

A, B), are 3–7-(majority 5-)septate with slight or no constrictions at the septa, are 25–67 × 7–14 μ (average 44 × 11 μ), slightly longer and narrower than in the Harkness description. The fungus reported by Neergaard (**9**) from Denmark varied only slightly from our collections; his spores were 2–6-septate, 42–66 × 10.5–15 μ (average 54.5 × 13 μ). Muriform conidia occurred sparsely in California material under very moist conditions on the host, prior to germination and in culture. These possessed at most only one or two longitudinal septa.

The fungus grows readily at laboratory temperatures on potato dextrose agar, forming a grayish white, cottony colony which darkens in the center and, in new isolates, forms a mass of light olive-green spores. The underside of the colony is dark green, no diffusable pigment being formed as in other *Heterosporium* spp. The perfect state was not found in nature, nor was it formed in culture.

LIFE HISTORY

Infection studies. Monoconidial isolates of the fungus were used to determine pathogenicity. A spore suspension of the fungus was atomized onto several plants grown outdoors; bell jars were put over the plants, pressed into the soil to maintain a humid atmosphere and shaded with a cloth screen. The jars were removed after three days. Checks sprayed with distilled water remained healthy. By the end of the fifth day minute reddish brown spots were visible on the stems and leaves of inoculated plants; these lesions later enlarged and became purple brown. By the tenth day many of the petioles had been completely girdled and the leaves had shriveled and died. The foliage which was not killed turned yellow. The calyptras were attacked in several cases (FIG. 1, A) as were the immature capsules, which became purple, shriveled, and dry. Several of the capsules tardily developed the char-

of petiole showing penetration and areas of gum formation. E. Cross section of leaf showing penetration peg. F–G. Appressoria-like organs and intercellular and subepidermal mycelium after 16–24 hours. H. Subepidermal penetration after 42 hours. I. Stomatal penetration. J. Composite drawing from 4-day-old inoculated material showing flattened, digitate subcuticular mycelium and narrow subepidermal mycelium.

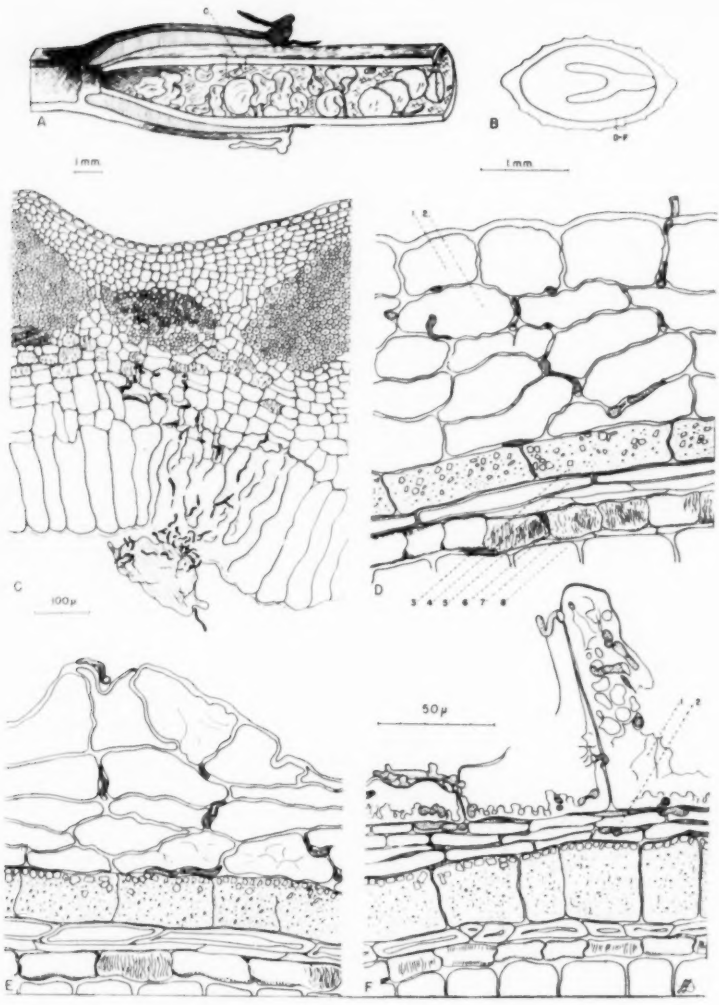

FIG. 3. Anatomical aspects of seed infection by *Heterosporium eschscholtziae*. A. Diagrammatic longitudinal section of capsule showing shrivelled seeds and darkened areas representing infection. Mycelium passes from receptacle into lumen of capsule through point of attachment. B. Cross section of seed showing region of sections D–F. C. Enlarged cross section of region from A, showing mycelium in capsule wall, necrotic area formed, and mycelium in rotted seed. D–F, Cross sections of seeds showing mycelium

acteristic elongated brown-black spots (FIG. 1, E). Other mature fruits which showed no symptoms or a slight stunting and a blackening of the receptacle and peduncle were found when split to have mycelium growing inside, which killed most of the seed (FIG. 3, C). The capsule in these instances acted as a moist chamber. The mycelium had entered the capsule at the point of its junction with the peduncle and receptacle (FIG. 3, A).

To determine the method of conidial penetration, leaves were removed from healthy plants, placed in Petri dishes containing moist filter paper, atomized with a spore suspension and held at laboratory temperatures. Epidermal strips were removed from the leaves at various intervals to determine the time and method of penetration. At the end of 2½ hours the conidia were germinating copiously but penetration had not occurred. About 10 hours later, a toxin reaction by the host occurred and by 16 hours, appressorium-like structures were sometimes evident and penetration was in progress (FIG. 2, F–G). The method of penetration was usually obscured by an abundance of dark brown, heavily staining gum formed at the point of entry (FIG. 2, D). The spore from which the infection arose was often still visible on the surface of young spots. Similar observations were made on epidermal strips from the four-day-old inoculated field material. Apparently the mycelium usually enters between epidermal cells, grows subcuticularly, then extends into subepidermal (FIG. 3, E, H, J), or mesophyll tissue. In a few cases, the mycelium penetrates directly through the cuticle. Stomatal penetration is extremely rare (FIG. 2, I). The subcuticular mycelium is flattened in comparison to the surface or subepidermal type and sometimes branches profusely, forming a broad band with "finger-like" projections (FIG. 2, J).

Zogg (**13**, **14**) described a similar host reaction to infection by *Pleospora calvescens* on opium poppy. He showed that the intercellular mycelium secreted toxins which stimulated the host to form a gummous demarcation zone between healthy and infected tissue. These deposits localized the fungus at temperatures below

in the layers of the seed coat. Very young seed shown in D, nearly mature seed in E, and dry mature seed in F. 1 = epidermis; 2 = parenchyma; 3 = crystal layer; 4 = fiber layer; 5 = cross cells; 6 = pigment layer; 7 = inner cells; 8 = endosperm.

16° C. and above 30° C., but did not kill it. He reported that at 18–20° C. a broad yellow-green area was formed, which was lacking at the optimum for the fungus (22–29° C.).

Germination time of the *Heterosporium* conidia on water agar varied between 1¼–1½ hours for spores from a fresh culture or high humidity, to 1½–2 hours for dry or old conidia (FIG. 2, C). Any of the cells of the spore were able to send out a germ tube.

Seed transmission. In order to determine whether the fungus was seed-borne, seed from infected capsules was removed aseptically and placed on water agar in Petri dishes. Only 1 out of 100 seeds germinated, the rest rotted and became covered with copious sporulation.

Sections of young and mature seed were examined to determine whether the fungus was carried internally as well as on the surface. Some of the mycelium-free seed from young infected capsules apparently had been killed by toxins formed by adjacent hyphae, while others showed the mycelium ramifying all through the developing seed coat layers. In most cases, the end of the seed projecting into the lumen of the capsule was the most heavily attacked, with the mycelium occurring along practically every cell wall. Dead embryos were found only in immature seeds. In green seeds (FIG. 3, D), the mycelium ramifies through the outer parenchymatous layers into the forming crystal layer,[2] many times disorganizing it and preventing its formation, then through the fiber, cross, pigment and inner cells on into the embryo. After the cells of the crystal layer are mature, they apparently constitute a barrier through which only occasional strands of mycelium pass. Consequently, in mature seeds the fungus is generally limited to the epidermis and to the layers of compressed parenchyma (FIG. 3, F).

CONTROL

Since the pathogen was found to be internally and externally seed-borne, hot-water treatment seemed the simplest and most efficient

[2] The names assigned to the various seed coat layers correspond to those of the opium poppy on which much work has been done (**15**, p. 430). The assistance of Dr. F. M. Scott and Katharine C. Baker in determining the layers in the healthy seed coat is gratefully acknowledged.

way of ridding the seed of the fungus. Trials were conducted with 3 lots of commercial seed to determine the effectiveness of such a treatment. Soaking the seed for ½ hour in water at 50° C. (122° F.) was found to eliminate the organism. Apparently germination was reduced 27%, but this figure is approximate due to erratic germination and dormancy which characterizes seed of this plant.[3] Treatment at 51.5° C. (125° F.) for ½ hour also killed the organism but gave 35% germination reduction.

This disease is important only in commercial seed plantings. Since the increased rate of seeding in such plantings to offset germination reduction is economically unimportant the higher temperature should be used to insure eradication. Germination tests should be run on seed lots treated to determine the proper rate of sowing necessary for a desired stand. Treated seed should be planted in land that has been free of California poppy for two years. To reduce chance of reinfection, plantings should not be made in proximity to a stand of wild or volunteer plants of this species.

SUMMARY

Although *Heterosporium eschscholtziae* Hark. has been known in California since 1884, it did not cause economic loss until 1947 when severe yield reduction in commercial seed fields occurred.

The disease may cause death of seedlings, or lesions on leaves, stems, roots, receptacles, calyptras, and fruit. Mycelium may penetrate the capsule at its point of attachment and copiously ramify through the interior of the fruit, killing young seeds, either adhering to the surface or penetrating the coats of mature seeds. The fungus thus becomes both externally and internally seed-borne.

An efficient method of eliminating the fungus from the seed is a hot-water treatment at 51.5° C. (125° F.) for ½ hour.

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[3] Information kindly supplied by Miss Betty Ransom of Ransom Seed Laboratory, Los Angeles, California.

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THE EFFECT OF CERTAIN PHENOLIC COMPOUNDS ON THE GERMINATION AND GROWTH OF MICROFUNGI

BERNARD VERDCOURT

(WITH 7 FIGURES)

Since the publication of the effect of sodium pentachlorphenate on the germination of *Aspergillus niger* Van Tieghem (Armitage and Verdcourt, Research **1**: 236–237. 1948), certain other similar observations have been made. A summary of these has already been published (Verdcourt, Trans. British Myc. Soc. **33**: 323. 1951) and this present paper forms an expanded account of these observations.

No specific references to these effects have been found in the literature though abnormalities of various kinds have been reported mostly in the absence of any apparent external stimulus. Schramm (Myc. Centralbl. **5**: 20–27. 1914) kept a degenerate colony of *A. niger* going which was entirely non-sporulating and reproduced vegetatively by means of sprout mycelia. The latter have very often been reported in species of *Mucor*. These degenerate forms produce colonies rather similar to those formed by yeasts. Vincent (Jour. Soc. Chem. Ind. **66**: 154. 1947) mentions that hyphal tips are thickened and distorted in the presence of methylhydroxybenzoic acid. Brian *et al.* (Trans. British Myc. Soc. **29**: 173–187. 1946) discovered that a substance produced by *Penicillium Janczewskii* Zal., later shown to be griseofulvin, had a marked effect on the germination of *Botrytis alii* Munn. The germ tubes become very swollen and distorted and growth then stops. Their figures show some swelling of the spores and are similar to the effect produced by sodium pentachlorphenate on *A. niger* at concentrations just below those necessary to give giant cells, only in the latter case a normal colony is eventually formed. They also give references to work describing morphological changes in fungi caused by metabolic products. The writer has found that griseofulvin allows

normal growth of *A. niger* even at concentrations of 20 ppm. What appear to be similar phenomena are mentioned by Gottlieb (Bot. Rev. **16**: 236. 1950) as occurring when *Rhizopus nigricans* germinates in the presence of 50% carbon dioxide. The giant cells described in this paper are superficially not unlike Hülle cells. Hülle cells, which are produced by many species of *Aspergillus*, are produced by hyphae as a result of some internal stimulus, whereas giant cells are produced by spores or germ tubes under the influence of, at any rate, a primarily external stimulus.

A brief description of the phenomenon which will be termed the "giant cell effect" will now be given. During normal germination a spore may swell to double its resting size and a narrow cylindrical germ-tube emerges through a small break in the spore wall (FIG. 1). The formation of giant cells is, however, preceded by a swelling of the spore to much more than double its original size. The spore coat stretches and at certain concentrations bursts and a cell emerges. The spore wall forms a persistent cap on the cell. The giant cell is thus a very thick and distorted germ-tube, the usual controls on its size and shape having been completely upset by an external chemical stimulus. The nuclear material increases very largely and squeezes out of the swollen spore into the cell. Later this material usually contracts to form a large granular nucleus. Eventually abnormal hyphae may be formed, but these are much larger than usual. At higher concentrations spores merely swell and no break occurs in the wall, i.e., formation of a germ-tube has been completely inhibited. The giant cell effect is subject to variation according to the concentration and chemical structure of the inhibitor and the species of mould under test. With some substances the spores swell to a moderate size and then germinate, the germ-tubes often being thick and branched. At any one concentration all the spores in one inoculation will not show the same behaviour. Some will not show any signs of change, others will germinate almost normally, and the rest will develop into giant cells. These, however, will vary in size and also in the degree of contraction of the nuclear contents. This is correlated with some properties of the spore which vary very considerably from spore to spore. Thickness, elasticity, and permeability of the spore wall are the most likely factors. Moreover, spores from one conidio-

phore vary considerably in age and size, and it is well known that poisons act quantitatively. In all cases it has been found that the effect on the morphology of the mould is only a temporary one and no lasting change is suffered by the genetic factors. If the external chemical stimulus is removed by transferring portions of the abnormalities to ordinary culture medium perfectly normal colonies result.

The effects have been studied by inoculating plates of Czapek-Dox agar containing known amounts of inhibitor with a liberal quantity of spores in order that enough would be available to lift several hundred out each day for examination. The pH of the medium is 4–5 and the small traces of inhibitors used have not appreciably altered this figure. The plates were incubated at 26° C. and examined daily under the low power of a microscope. If any signs of change were apparent spores were lifted out and mounted in lacto-phenol-chlorazol black E mixture. Throughout this paper when an "x ppm. y agar" is mentioned it refers to a Czapek-Dox agar containing x ppm. of the inhibitor y.

Giant cell effects have so far been observed only in the presence of inhibitors having a straight phenolic group, or one in which the hydrogen of the group has been replaced by an alkali metal. Very small cells have, however, been obtained with agar containing 70,000 ppm. of ethyl alcohol. They have not been noted in the presence of inorganic poisons such as copper sulphate, potassium dichromate, or thallium salts; nor have they been observed in the case of non-phenolic organic inhibitors, or the phenyl mercury compounds. When such non-phenolic inhibitors are incorporated in the culture medium the rate of colony growth of the fungus is reduced further and further as the concentration is increased. At a certain concentration spores fail to germinate, but until this concentration is reached spores germinate almost normally, there being no intermediate abnormal phase. The inhibition concentration is not absolutely critical; the percentage of spores that germinate decreases as the concentration increases since the individual susceptibility of spores varies over a range.

The fungus chiefly employed during these investigations has been *A. niger*, but some observations have also been made on *Peni-*

cillium chrysogenum Thom and certain other moulds. The effects will be dealt with under chemical headings.

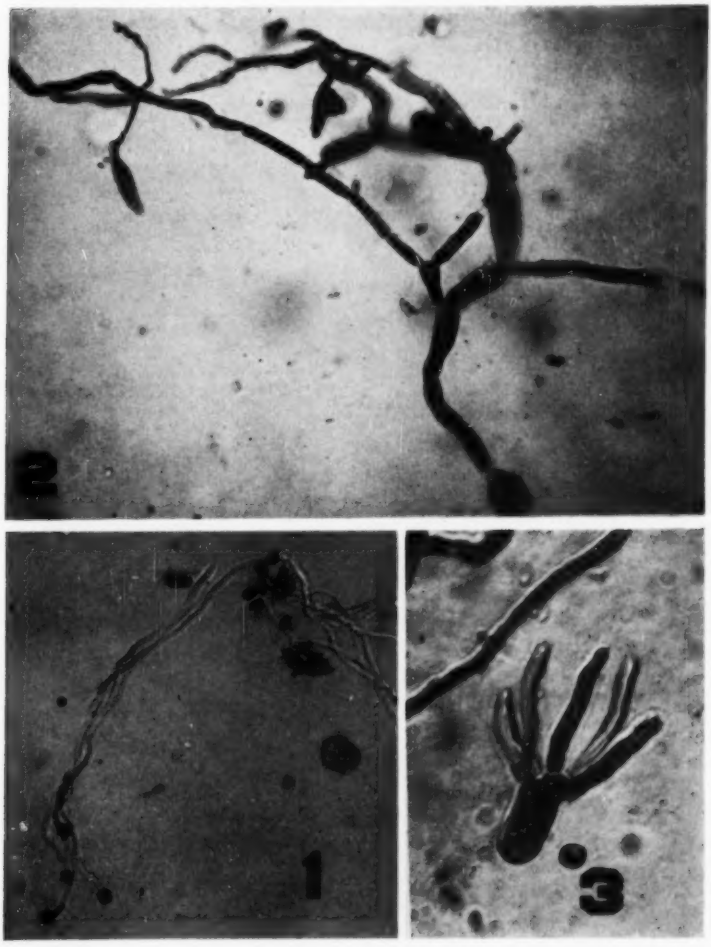
PHENOL

1,000 ppm. of phenol inhibits the growth of *A. niger* altogether but on a 750 ppm. agar most spores swell up to form large giant cells but some germinate forming abnormal hyphae (FIG. 2). *Penicillium* spores also form small giant cells on 750 ppm. agar but these rapidly germinate. An abnormal growth, together with giant cells, was obtained as a contaminant on 1,000 ppm. agar and these on sub-culturing gave rise to a non-sporulating species of *Mucor*. At lower concentrations around 500 ppm. fairly normal growth of *A. niger* was obtained but conidiophores bore very distorted sterigmata in some instances.

SUBSTITUTED PHENOLS

The introduction of one or two chloro or nitro groups into the phenol molecule greatly increases its toxicity. With five chloro groups the toxicity is high. Dinitrophenols are very toxic but s-trinitrophenol (picric acid) is less toxic than phenol itself. The explanation for this may rest in the fact that it is known that anions of organic acids penetrate cell membranes less readily than un-ionized molecules. Phenol itself is a very weak acid ($k = 1.3 \times 10^{-10}$) and as nitro groups are substituted so the toxicity of the un-ionized molecule increases but also the dissociation constant increases. Picric acid is a strong acid ($K = 1.6 \times 10^{-1}$) and this probably counterbalances the increased toxicity which the introduction of three nitro groups would lead one to expect. Somewhere between phenol and trinitrophenol one would expect a compound with the greatest toxicity of any of the series. Dinitrophenol would appear to be this compound. S-tribromophenol on the other hand is a very effective inhibitor. The introduction of further hydroxy groups into the phenol molecule greatly reduces the toxicity. For example *A. niger* will grow quite normally, though slowly, on 7,500 resorcinol agar, and on 4,000 ppm. phloroglucinol agar. A substituted amino group such as occurs in metol also reduces the toxicity and *A. niger* will grow on a 5,000 ppm. metol agar. Alkyl

groups increase the toxicity but ethers of phenols and substituted phenols have a lower toxicity than the phenols themselves. For example, 200 ppm. p-chlorophenol will inhibit *A. niger,* but 1,000 ppm. of p-chlorophenyl a-glycerol ether is needed for inhibition.



FIGS. 1–3. 1. Normal germination of *Aspergillus niger* spores on uninhibited agar, × 365. 2. Abnormal germination of *A. niger* on 750 ppm. phenol, × 365. 3. Abnormal germination of *A. niger* on proprietary chlorphenol, × 645.

A table of approximate inhibiting values for *A. niger* follows and a phenyl mercury derivative is included for comparison.

Large giant cells have been noted when *A. niger* is inoculated onto 180 ppm. thymol agar and 10 ppm. s-tribromophenol agar and smaller ones are usually formed on 50 ppm. 2:4 di-chlorophenol agar. In both cases fairly normal colonies resulted in about three weeks, possibly due to the volatile nature of the compounds. *Penicillium chrysogenum* produced a very abnormal white non-sporulating colony on 250 ppm. thymol agar and green "wedding cake" type colonies on 180 ppm. agar.

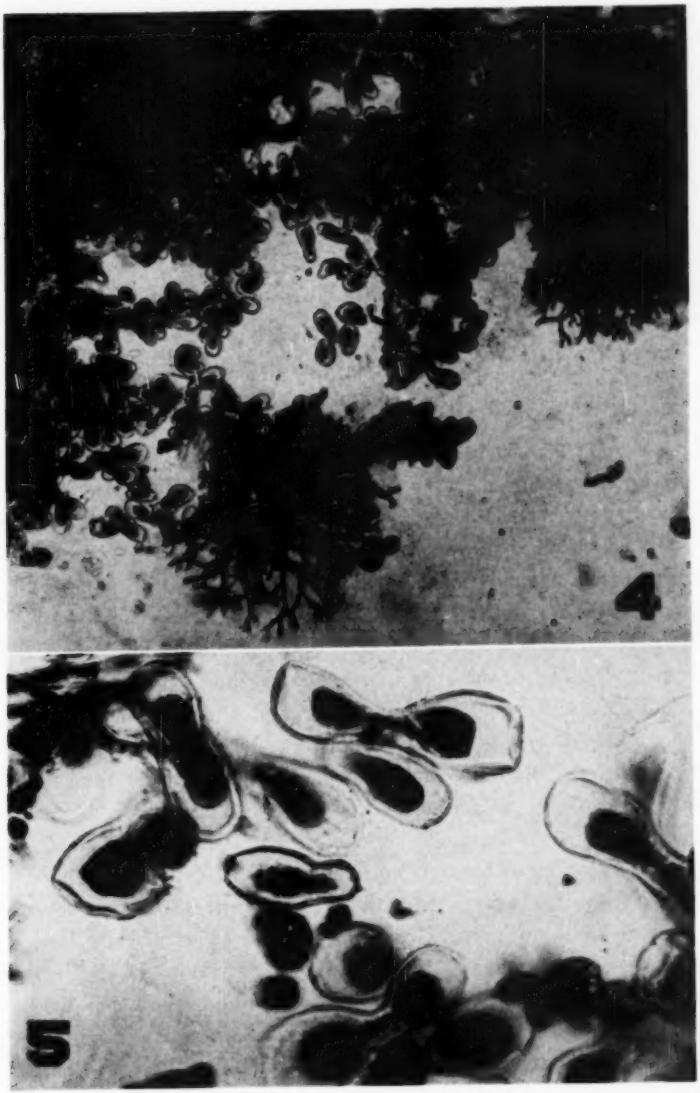
TABLE OF INHIBITING CONCENTRATIONS OF CERTAIN PHENOLS ON *A. niger*

Substance	Concentration
Phenol	1,000 ppm.
o-nitrophenol	125
m-nitrophenol	500
p-nitrophenol	250
p-chlorophenol	200
2:4 dichlorophenol	200
s-tribromophenol	20
thymol	250
sodium pentachlorphenate	100
2:4 dinitrophenol	50
s-trinitrophenol	1,200
sodium o-phenyl phenate	200
p-chlor-m-cresol	100
phenyl mercury acetate	5

Giant cells have not been noted on any of the nitrophenols though the concentrations needed may be very critical. A complete range of concentrations of picric acid was, however, tested and no giant cell formation was at all apparent at any concentration.

Giant cells have also been obtained with various proprietary chlorophenols and an exceptionally odd phenomenon was observed while testing an agar containing 30 ppm. of such a compound. Spores swelled and produced sausage-shaped giant cells which later sent out germ-tubes. The number of germ-tubes may be as high as eight and an extreme instance of this "octopus effect" is shown in FIG. 3.

A slight modification of the giant cell effect has been noticed with 100 ppm. sodium-o-phenyl phenate agar. Spores in general swell but little, and the germ-tubes after emerging form spherical cells.

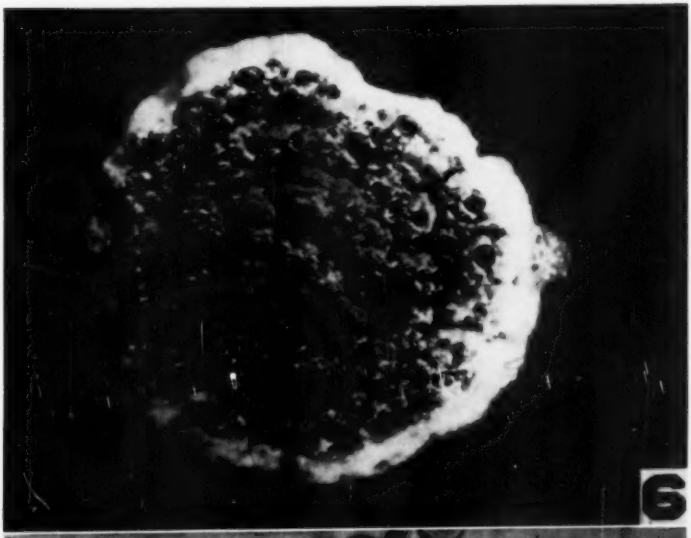


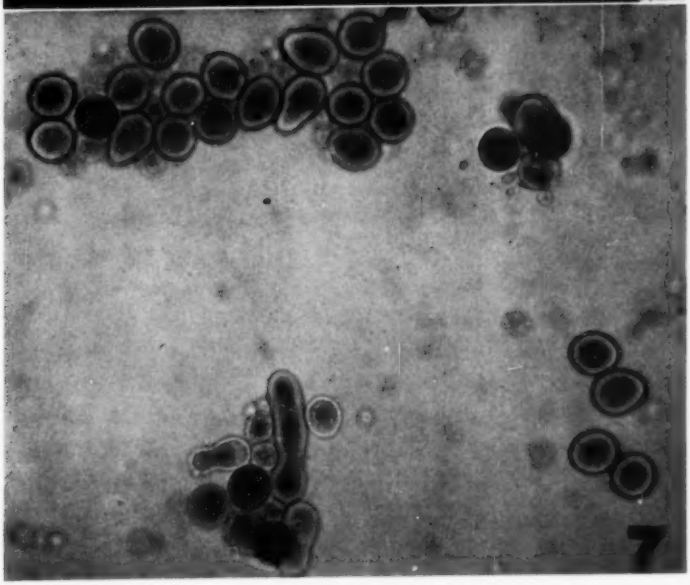
FIGS. 4–5. Giant cells of *A. niger* on 30 ppm. sodium pentachlorphenate. 4. × 130. 5. × 600.

This type of behavior is occasionally observed with sodium pentachlorphenate but it is not the rule.

The effect of sodium pentachlorphenate has been studied more than that of any other substance and the general nature of its action on *A. niger* has already been described (Armitage and Verdcourt, **1948**). At concentrations of 25–40 ppm. the spores swell and their walls stretch and break allowing the cells to emerge in 3–4 days. The spore wall remains as a sort of cap but this is easily visible only when the cells are mounted in a medium of low refractive index. Sporulating colonies are sometimes eventually produced.

The following experiment carried out on agars within this range of concentrations demonstrates clearly that the phenomenon is linked up with the unbroken spore wall. Spores of *A. niger* were incubated on a normal Czapek-Dox agar, and a portion of this agar containing germinating spores showing long germ-tubes together with some unchanged spores was removed after 18 hours and placed on a 25 ppm. sodium pentachlorphenate agar. Within four days many of the originally unchanged spores had swollen to form characteristic giant cells. The germinating spores, however, showed no swelling of any kind either in the spore or the germ-tube. The germ-tube, however, had continued to grow in an extremely zig-zag and distorted manner quite unlike the straight growths normally obtained. At 50 ppm. spores swell but the spore wall does not usually break. At these higher concentrations growth is halted at the giant cell stage, and colonies are not formed. It was noted that after about a month in contact with a 100 ppm. agar a very small percentage of spores undergo a slight swelling. This is, moreover, quite marked, since the nuclear contents often condense to form a small nucleus as they do in the case of larger cells formed at lower concentrations. Some further illustrations of the larger cells are given (FIGS. 4–5). Precisely similar giant cell effects have been noticed with the closely allied *A. japonica* Saito. The behavior of *P. chrysogenum* is rather similar to that of *A. niger* but the concentrations needed to produce giant cells are much higher. Up to about 20 ppm., growth is quite normal but at 25–50 ppm. colonies are slow-growing, fasciculate, and sometimes coremiform. They are in their early stages very reminiscent of small





FIGS. 6–7. 6. "Wedding cake" type colony of *Penicillium chrysogenum* on 30 ppm. sodium pentachlorphenate, × 4. 7. Giant cells of *P. chrysogenum* on 100 ppm. sodium pentachlorphenate, × 580.

wedding cakes (FIG. 6). At 50 ppm. spore germination may be delayed by as much as 40 hours and at 100 ppm. most spores form giant cells but some germinate almost normally (FIG. 7). 150 ppm. inhibits all growth. Some very much branched hyphal growths of *A. niger* were obtained by inoculating spores on 150 ppm. sodium pentachlorphenate agar containing some animal charcoal. The latter adsorbed the inhibitor and depressed the concentration irregularly over the plate.

NAPHTHOLS

Both α- and β-naphthol are efficient inhibitors, 120 ppm. of the latter and 200 ppm. of the former being sufficient to prevent the germination of *A. niger* spores. Large giant cells develop on 80 ppm. β-naphthol agar, some of the cells being over ten times the diameter of a normal spore. A small percentage of spores, however, germinate fairly normally. Slight swelling of spores, which later germinate abnormally, occurs on agar containing 80 ppm. of α-naphthol and a rather similar effect was noted on 100 ppm. α-naphthylamine agar.

The writer is indebted to F. D. Armitage, Esq., for assistance with the work.

SOMMERSTORFFIA SPINOSA ARNAUDOW

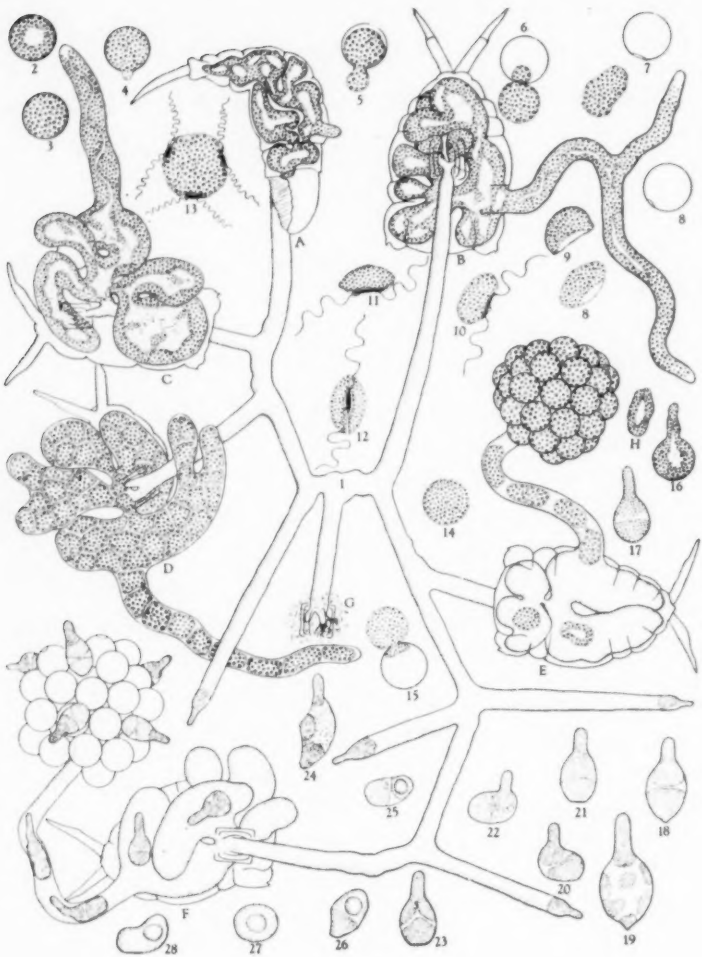
JOHN S. KARLING

(WITH 76 FIGURES)

Most of the fungi which belong to the family Saprolegniaceae are saprophytes, but a few members of the genera *Aphanomyces, Synchaetophagus, Hydatinophagus* and *Sommerstorffia* parasitize microscopic animals. Of these species *Sommerstorffia spinosa* is unique in that it is the only known member of this family which has developed highly specialized vegetative structures for the capture of its prey.

This fungus was first observed by Arnaudow (**1923**) in Sofia, Bulgaria, parasitizing species of the rotifer genus *Monostyla*, and six years later it was reported again by Sparrow (**1929**) in the same hosts from Cambridge, Mass. These are the only two published reports of its occurrence as far as the writer is aware, and from these accounts the fungus appears to be quite rare in nature. However, the author has found a few specimens of it in soil from New Jersey (**1936**), Virginia (**1939**), Louisiana (**1947**), and recently (**1950**) in Alaska, which indicates that it may be widespread in distribution. Nevertheless, its non-fungus-like appearance in the vegetative stage, relatively small size, and sparseness when rotifers are not abundant make it inconspicuous, and unless the observer is already familiar with the fungus it may be easily overlooked.

Arnaudow gave a description of its predaceous habit, vegetative structure, asexual reproduction, parthenogenic oospores, and taxonomy in two papers which were accompanied by five text-figures. Sparrow confirmed Arnaudow's observations on the parasite's vegetative structure and asexual reproduction, but, apparently because of a scarcity of material, was unable to add anything further to our knowledge of this fungus except to extend its range of distribution to North America. Accordingly, not very much is known about the host range of this fungus, its method of zoosporo-



FIGS. 1–28.

FIGS. 1–28. *Sommerstorffia spinosa*. 1. Extensive thallus with eleven branches and six captive specimens of *Distyla* sp. 1A, 1B, Developmental stages of thallus and sporangium in the rotifer's body. 1D. Zoospore initials in sporangium and exit tube. 1E. Cystospore clusters at orifice of exit tube. 1F. Empty sporangium and cystospore vesicles; a few cystospores have developed directly into sporelings. 1G. Remnants of mouth parts of the first captured rotifer. 1H. Cleavage segment showing central vacuole and coarsely granular protoplasm. 2, 3. Median and surface views, respectively, of cysto-

genesis, structure and behavior of its zoospores, development of the thallus, and oospore formation. The present study was undertaken to elucidate these processes as far as possible and thus supplement the observations of Arnaudow and Sparrow.

The fungus used in this study occurred in wet soil which was collected by Betty J. Locker in August, 1950, in an alpine tundra stream at an altitude of 6500 ft. in the Talkeetna Mountains (lat. 62° N., long. 149° W.) in Alaska. After arriving at Purdue University, the soil was covered with animal charcoal water and baited with thin strips of chitin and onion skin. In the course of several weeks numerous rotifers appear in the culture, and several of these were caught by and became infected with *S. spinosa,* which apparently was attached to the floating strips of chitin and onion skin. These strips were then collected and added to another watered soil culture with an abundance of rotifers, and in this manner the thalli of *S. spinosa* were concentrated. These multiplied rapidly by zoospores, and within a few days a fairly dense culture of the fungus was established. It was maintained without much difficulty by transferring thalli to new cultures of rotifers every eight days. *Sommerstorffia spinosa* derives its nourishment from captured rotifers, and all attempts so far to grow it on synthetic media were unsuccessful. Therefore, to maintain this fungus in culture for an extended period of time it is necessary to maintain a constant supply of rotifers. The rotifers used in this study were obtained from a biological supply company and grown in large 12 in. diameter finger bowls in approximately 2½ inches of animal charcoal water on the surface of which were floated numerous strips of thin onion skin. This substratum provides excellent food

spores. 4–8. Stages of germination of cystospore and emergence of rudimentary zoospore. 9. Stage in which the flagella and ventral furrow appear, which initiates the "rocking" movement of the zoospore. 10. Mature zoospore at end of "rocking" period. 11, 12. Side and ventral views, respectively, of mature zoospore. 13. Large octoflagellate zoospore. 14. Zoospore after coming to rest. 15. Second emergence stage of rudimentary zoospore. 16, 17. Germination of zoospore and its development into a sporeling, respectively. 18–24. Longitudinal views of sporelings, showing clear equatorial band and separation of the protoplasm into apical and basal portions of refractive substance. 25–28. Surface views of sporelings; neck appears as a highly refractive globule.

for the rotifers and also a place for the zoospores of the fungus to come to rest and develop into sporelings. Such infested pieces of onion skin were then transferred to new cultures of rotifers, and by following this procedure the present culture of *S. spinosa* has been maintained for more than a year.

VEGETATIVE STRUCTURE

As described by Arnaudow the vegetative thallus of *S. spinosa* is limited in length and consists of 1 to 6 or rarely more, straight or slightly bent, 100–150 μ long by 8 μ wide, tubular, non-septate branches, which are terminated by a narrow peg or blunt spine filled with homogeneous, highly refractive substance. The present observations confirm those of Arnaudow in general with only minor variations and additions. In the Alaskan material some of the thalli were quite large (FIG. 1), and in exceptional cases extended for a distance of 680 μ. The majority of thalli, however, were less extensive and bore only 3 to 6 branches. The latter are distinctly tubular, taper slightly, and vary from 5.6 to 10.4 μ in diameter, with an average of 7.9 μ. In very old cultures, on the other hand, the branches may sometimes be as much as 15 μ in diameter in local regions, somewhat gnarled in appearance and quite irregular in outline. As is readily demonstrable by microdissection, the branches are comparatively rigid and generally have the appearance of slightly tapered, pointed and stiff tubular spikes. This rigidity and the obtuse or almost right-angle habit of branching give the thallus a non-fungus-like aspect. In the vegetative stage, when the thallus is not feeding on a rotifer, the protoplasm in the branches is sparse, finely granular or fairly homogeneous and highly vacuolate (FIGS. 1, 40–42) and does not appear as refractive as that in the tips.

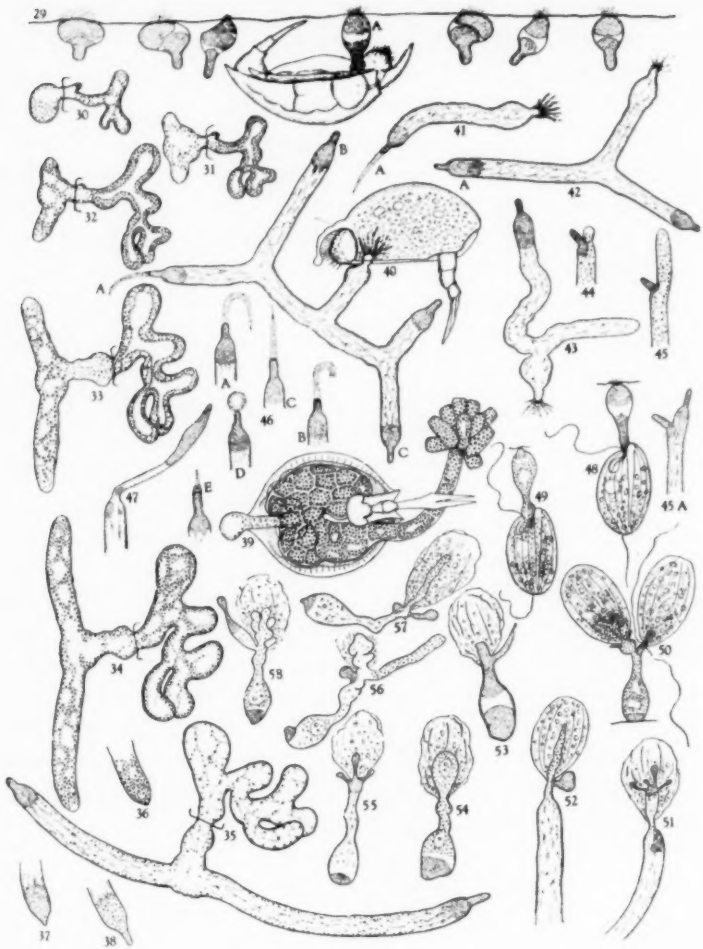
Particularly noteworthy are the abruptly tapered pegs or blunt spines at the ends of the branches. These vary from 6 to 8 μ in length and 3 to 5 μ in diameter, and are filled with a refractive substance. Arnaudow described this material as highly refractive, but in the Alaskan specimens it is not brilliantly gleaming but somewhat dully refractive. Nevertheless, it stands out quite sharply in contrast to the protoplasm in the branches. Usually it

extends back but a short distance into the tubular branches but in a few unusual cases it extended back as much as 25 μ. The lower boundary appears to be rounded in contour (FIGS. 1, 41, 42), but quite often it may be quite irregular, with strands or tendrils running down into or connecting with the protoplasm of the branches. These pegs or blunt spines are the specialized organs for capturing the rotifers, and the refractive substance within them probably is the active agent of prey. Frequently, the blunt spines or pegs bear an extension of hyaline, homogeneous substance which is usually curved or hook-like (FIGS. 40A, 41A, 46A, 46B). Occasionally, it may be straight (FIG. 46C), and in very rare instances globular (FIG. 46D). These extensions apparently were not observed by Arnaudow and Sparrow, but they are quite common in the Alaskan material. In a count of 175 thalli such extensions were present on ninety-five and lacking on eighty of the pegs. Furthermore, they may be present on one and lacking on another peg of the same thallus (FIG. 40). Similar extensions were observed earlier in the New Jersey, Virginia and Louisiana material.

The significance and purpose of these extensions are not known. They are hyaline and homogeneous in appearance and not distinctly refractive like the substance within the blunt spines. Nor are they adhesive to a microdissection needle or passing rotifers and other microscopic animals. Nevertheless, they are fairly rigid, and it is very difficult to straighten them out with a needle. They might possibly be exudations of the protoplasm within the pegs, but it is to be noted that they lack the high refractive index of the latter.

DEVELOPMENT OF THE THALLUS

After coming to rest on the under surface of the floating substratum the zoospores round up (FIG. 14) and develop into sporelings within 4 to 14 hours. The first step in this process is the development of a germ tube which is quite broad, relatively short and slightly tapered (FIG. 16). In this stage the protoplasm is still very coarsely granular with a central vacuole and looks like that of the active zoospore. Within a few hours, however, it gradually becomes more finely granular (FIG. 17), and this change continues until the protoplasm appears almost homogeneously



FIGS. 29–58.

FIGS. 29–58. *Sommerstorffia spinosa*. 29. Schematic drawing showing sporelings attached to and hanging down from the lower surface of floating opinion skin into the water; rotifer caught on neck of sporeling at A. 30–35. Successive stages of the endo- and epibiotic development of a thallus from a sporeling; rotifer body omitted. 36–38. Successive stages of the formation of a terminal spine or predaceous peg. 39. Completely endobiotic development of a thallus and sporangium. 40. Completely epibiotic development of a thallus; note dichotomy of branching. 41–43. Small epibiotic thalli. 44, 45.

refractive. Concomitant with these changes in appearance it usually separates into a basal and apical portion with the result that a clear biconcave zone or area extends across the lower portion of the sporeling (FIGS. 17, 18, 21–23). This zone usually stands out so sharply from the refractive protoplasm that it frequently looks like a broad transverse wall. Arnaudow apparently failed to observe this separation and the refractive character of the basal portion of the protoplasm, because it is not shown in his figures of the sporelings. Occasionally in large sporelings, the optically heterogeneous protoplasm seems to aggregate in masses and become transformed into islands of refractive substance (FIGS. 19 and 24). The sporelings shown in these two figures developed from octo- and tetraflagellate zoospores, respectively.

The majority of mature sporelings have the shape of tenpins or flasks with a neck which is approximately one-half as long as the diameter of the swollen basal portion. The base may be flattened (FIGS. 21, 23) or slightly pointed (FIGS. 18, 19). Fairly often, the sporelings may have the shapes shown in FIGS. 20 and 22. They vary from 6.8 to 12.2 μ in greatest diameter by 13.6 to 20.4 μ in height. Exceptional sporelings such as shown in FIG. 19 may be 20.5 μ in diameter and 45.9 μ in height. In surface or top view they are globular (FIG. 27), elongate (FIG. 25), slightly angular (FIG. 26) or curved in outline, with the narrower neck appearing as a highly refractive globule.

The sporelings adhere to and hang down from the under surface of the substratum (FIG. 29). They appear to be strongly stuck to the latter, and it is extremely difficult to dislodge them mechanically by dissection without tearing the substratum. Nor are the rotifers which they capture able to pull them off. It is not certain whether or not the refractive material in the base is the adhesive agent, but it may be noted in this connection that the sporelings do not develop

Stages in the resumption of growth of branches beyond predaceous peg. 45A. Second predaceous peg formed shortly after resumption of growth. 46A–46E. Variations in shape of hyaline matrix at end of predaceous pegs. 47. Unusual extension of predaceous peg. 48–50. Specimens of *Entosiphon ovatum* captured by sporelings. 51, 52. Modifications of predaceous pegs which have captured *E. ovatum*. 53–58. Variations in size and shape of sporelings which have captured and fed upon *E. ovatum*.

structurally distinguishable holdfasts which might anchor them to the substratum. In large rich cultures of *S. spinosa* hundreds and even thousands of sporelings may be found on the under surface of floating pieces of onion skin, and as the rotifers feed along this surface they are caught on the tapering neck or tip of the sporelings (FIG. 29A). These tips are, accordingly, as effective in capturing prey as the predaceous spines or pegs on the mature thallus, and in no cases observed were the rotifers able to pull away or free themselves from the sporelings. Quite probably, some of them do but we have not observed it. In one experiment, a sporeling-infested, 3 × 6 mm., strip of onion skin was added to a rich culture of *Monostyla* sp. and on examination 6 hours later 860 captured rotifers were counted on this strip. From repeated observations of this kind it has become evident that as many or more rotifers are captured by sporelings as by the mature thalli. Sporelings may remain potentially predaceous for 8 to 12 days, but unless they capture a rotifer by that time they begin to degenerate. Obviously, their continued existence and development depend on the presence of a suitable host.

It should be noted here that the zoospores may come to rest on any type of substratum and develop into sporelings. Accordingly, sporelings may be found attached to the mycelium of other fungi, i.e., *Rhizopus, Saprolegnia, Achlya, Penicillium, Aspergillus, Fusarum, Alternaria,* etc., which may be growing in the cultures. Rotifers caught on such attached sporeling may bend or drag the mycelia back and forth in the struggle to free themselves. If a culture dish is left uncovered or exposed for several days usually a thin film will form on the surface of the water. The zoospores may come to rest on its under surface and develop into sporelings which then hang down into the water. Rotifers caught on such sporelings are able to pull them loose from the film and go swimming away with the sporeling attached to their mouth. Such rotifers, however, are incapable of feeding, although their cilia may beat for several hours, and are eventually killed by the developing fungus.

Following capture of its prey the sporeling resumes growth and gives rise to the thallus. The development of the latter varies considerably in relation to its host, and for convenience it may be

classified as completely endobiotic, epibiotic, or endo-epibiotic. These types of development are not always sharply delimited and may merge into each other, but for purposes of description they may be designated as such. In the so-called endo-epibiotic type a broad germ tube or branch develops from the tip of the sporeling and grows into the deeper part of the rotifer body where it branches, enlarges, becomes deeply-lobed and contorted and eventually fills the body of its host. Successive stages of this type of development are shown in FIGS. 30–35. In these illustrations the body of the rotifer has been omitted to save space, and a line or half bracket has been used to indicate which portions of the developing thallus are epi- and endobiotic. FIG. 30 shows a young stage in which the endobiotic part is branching and becoming lobed, while the epibiotic portion of the sporeling remains unchanged. Note that the homogeneously refractive protoplasm which is characteristic of sporelings has become more coarsely granular and vacuolate. A slightly later stage is shown in FIG. 31 in which the epibiotic part of the sporeling has enlarged and formed the rudiments of two opposite branches. These elongate as more and more nourishment is absorbed from the rotifer (FIGS. 32, 33), and as a result the external part of the thallus at this stage usually has the shape of a T or foot. In the meantime, the endobiotic part becomes more extended and deeply lobed. Particularly noteworthy in these stages is the presence of a conspicuous central vacuole (FIGS. 31–33) which is surrounded by a relatively thin layer of coarsely granular protoplasm. Frequently, the vacuole is so extensive and branched that the granular protoplasm appears to consist of floating islands connected by strands. This coarsely granular nature of the protoplasm is characteristic of the developing external branches also (FIGS. 33, 34). The granules are fairly large and uniform in size and have an olive-gray gleam which gives the protoplasm a characteristic darkish refringence.

FIG. 35 shows a late and almost mature stage of development. The endobiotic development has been completed and most of the protoplasm has passed out into and been used up in the development of the external branches. Eventually the endobiotic part of the thallus becomes almost completely empty. The external branches in FIG. 35 have elongated considerably and developed terminal

predaceous pegs which are filled with the characteristic refractive material described elsewhere. These pegs are now capable of capturing rotifers, and the thallus may be regarded as mature.

The development of the predaceous pegs and the concomitant changes in the appearance of the protoplasm occur in a characteristic manner and may be followed readily. As shown in FIG. 34, the ends of the growing branches are bluntly or only slightly tapered and their tips are usually hyaline, but after the branches have attained definitive lengths they begin to taper abruptly (FIG. 36). Then a small tip begins to form (FIG. 37) which gradually develops into the characteristic peg (FIG. 38). While this is going on the granular protoplasm accumulates in the tip (FIG. 36), gradually becomes more finely granular (FIG. 37), and finally takes on the more or less optically homogeneous and refractive appearance (FIG. 38).

The endo-epibiotic type of development described above in which no reproductive structure develops within the body of the rotifer is not very common. More often the endobiotic portion will develop into a sporangium with an exit tube and form zoospores at the same time that the external branches are developing. Thus, thalli as well as zoospores are formed for multiplication and distribution of the fungus.

It is to be noted in the early developmental stages (FIGS. 31–35) that the thallus of *S. spinosa* branches dichotomously. This may continue until four equal branches are formed (FIG. 40). However, by that time the branches have usually developed predaceous pegs and captured rotifers which leads to unequal branching and suppression of dichotomy. Thalli with eight equal branches are extremly rare. After a mature thallus has captured a rotifer and begun to draw nourishment from it, the branches may resume growth, whereby the thallus becomes more extensive. In this process the protoplasm in the branches which resume growth becomes more abundant and coarsely granular. At the same time a bud forms at the base of the predaceous peg (FIG. 44) and gradually develops into an extension of the branch (FIG. 45). The original predaceous peg remains at the side of the branch, and eventually another develops at the tip of the extension. Sometimes, the extension of the branch may be very short and develops

a peg almost immediately (FIG. 45A). In this manner the thallus may become very extensive with a large number of predaceous pegs. The largest thallus so far observed had 23 captive rotifers in various stages of degeneration. In such cases the branches and captured rotifers may appear like a snarl of hyphae, as described by Arnaudow.

Completely endobiotic development occurs occasionally, but it is not very common according to our observations. In this type the tip of the sporeling branches and gives rise to an irregular, deeply-lobed structure in the manner described above for the endo-epibiotic method. However, there is no external development of the sporeling, and all that is visible outside of the rotifer's body is the globular base of the sporeling (FIG. 39). The endobiotic part of the thallus fills the body of the rotifer, and at maturity becomes a zoosporangium. After an exit tube has grown out through the posterior end of the host, the protoplasm undergoes cleavage into zoospore initials or rudiments (FIG. 39), and after the latter have escaped and dispersed, the remnant of the thallus slowly degenerates. Accordingly, such thalli are holocarpic and are used up in the production of zoospores.

Thalli which developed entirely epibiotically are shown in FIGS. 40 to 43. In such cases the tip or neck of the sporeling does not branch and grow within the body of the rotifer but functions primarily as an absorbing organ after capturing its prey. After the captive rotifer dies its internal organs begin to disintegrate and are slowly absorbed by the developing sporeling. In a short time the globular base of the latter elongates, becomes tubular and forms two opposite branches as described previously. These may branch very soon and form two secondary branches at right angles to them as shown in FIG. 40. Frequently, however, the two primary branches may form predaceous pegs (FIG. 42) which usually stops further branching until another rotifer is captured. In exceptional cases the elongated sporeling base does not branch but merely forms a predaceous peg at its tip (FIG. 41). Occasionally, in other thalli, one of the branches may cease to grow and ends bluntly without branching or forming a predaceous peg (FIG. 42). Such branches may be found on more extensive thalli also, particularly in old cultures. The part of the thallus immediately out-

side of the rotifer is usually slightly inflated, and this swelling is part of the bulbous base of the sporeling from which the extramatical thallus developed. It is to be noted in the completely epibiotic type of development that there is no growth of the fungus within the host. However, irregular and poorly-defined, coarse rhizoid- or root-like extensions often may be found radiating from the base of the thallus (FIG. 40). These may be mistaken readily for holdfasts or absorbing organs, particularly if the thallus becomes separated from the degenerated rotifer (FIGS. 41–42), but careful maceration and dissection studies reveal that they are the remnants of the host's mouth parts and other protoplasmic debris which have adhered to the tip of the sporeling. As noted elsewhere no structurally distinguishable and specialized holdfasts or absorbing organs have been found at the base of the thallus, and in the epibiotic type of development the tip of the sporeling serves both as an absorbing and capturing organ.

It may be pointed out here that the sporelings of *S. spinosa* may capture *Entosiphon ovatum,* a protozoan, in addition to rotifers. To date more than 500 such captured specimens of this species have been counted. After the protozoan is caught at the tip of the sporeling (FIG. 48) its two flagella beat and lash about, and the small animal uses all possible means of freeing itself without success. The struggle may continue for one to four hours, and gradually the lashing of the flagella ceases. In the meantime the tip of the sporeling usually branches and grows into the cytostome and body of the protozoan (FIG. 49). As it does so and begins to absorb food the protoplasm of the sporeling loses much of its homogeneous refringence and becomes more granular. At this and later stages (FIGS. 50, 54, 56, 58) one or more vacuoles appear which contain granules in active Brownian movement. The sporelings frequently develop refractive knobs and branches outside of the protozoan (FIGS. 53, 55, 56, 57) which are predaceous also, and occasionally a second protozoan may be caught on such knobs (FIG. 50). As a result of the capture of and feeding on *Entosiphon ovatum* the neck of the sporeling changes markedly in shape as shown in FIGS. 49, 50, 53–58. It may become swollen (FIG. 54), contorted (FIGS. 57, 58) and branch once to several times (FIGS. 56–58). In no case did zoosporangia develop in the pro-

tozoa. This is probably due to the fact that there is not sufficient food in the protozoa to support more extensive growth.

The predaceous pegs of mature thalli may occasionally capture *E. ovatum* also. So far only 38 cases have been observed, and in most of these the pegs (FIGS. 51, 52) had undergone much the same changes as the necks of the sporelings noted above.

SPOROGENESIS

Sommerstorffia spinosa reproduces asexually by the formation of zoospores as described by Arnaudow and Sparrow and shown in FIGS. 1E, 2 to 12. Arnaudow reported that differentiated sporangia are not developed in this species and for this and other reasons he placed it next to *Aphanomyces* in the Saprolegniaceae. In the Alaskan material, on the other hand, a few of the sporangia were markedly different in size and appearance from the remainder of the thallus, and therefore we shall describe them as sporangia with well-defined exit tubes. The processes of sporangium maturation, cleavage, and sporogenesis as well as the behavior and structure of the zoospores are fundamentally the same as in other species and genera of the family Saprolegniaceae. Nevertheless, they will be described in some detail here for the primary purpose of emphasizing the relationship of *S. spinosa* to this family. Various stages of the development and maturation of unusually large and broad sporangia are illustrated in FIG. 1 of an extensive thallus with six captive rotifers. As noted in the various types of development described earlier, the sporangia develop in the rotifer, and in no instance has a sporangium been observed to develop outside of the host. Quite often, however, sporangia may be found outside and completely free of the rotifer shell, but in our opinion this is the result of coverglass pressure and other disturbances in mounting the material for microscopic examination. The large and mature sporangium often distends and breaks open the rotifer shell with the result that the latter becomes loose and slips off when the specimens are mounted and the coverglass is applied. It is not improbable, on the other hand, that the sporangium may develop outside of the host in a well nourished culture, but so far we have not observed its occurrence.

In the early stages of development the incipient sporangium is very vacuolate, as related earlier. A central vacuole may extend into all lobes of the sporangium and occupy the greater part of its volume with only a thin surrounding layer of granular protoplasm. The early stage shown in FIG. 1A is exceptional in that only a few small vacuoles are present. The exit tube or tubes begin to develop fairly early and almost always protrude out of the posterior end of the rotifer (FIG. 1F). Very rarely do they grow through the shell as shown in FIG. 1A of a newly hatched or young captive rotifer. In mounting the specimens, however, loose shells may occasionally be shifted in position relative to the sporangium so that the exit tube appears to be emerging from the side. The tubes may grow to great lengths and in exceptional cases extend up to 680 μ. They may be straight, curved, coiled or contorted, and branch once to several times. Usually, the exit tubes are curved, vary from 50 to 160 μ in length, 8 to 12 μ in diameter at the base, and taper gradually towards the tip (FIGS. 1B, 1C, 1D). They may include several elongate vacuoles (FIG. 1B) which may be continuous with the central vacuole in the sporangium proper (1C). As noted earlier the sporangia usually fill the body of the host, are irregular in outline and deeply lobed, and rarely may be up to 20 μ in greatest diameter. The more optically heterogeneous protoplasm in the exit tubes as well as in the sporangia is very coarsely granular with an olive-greyish gleam and stands out in sharp contrast to that of the vegetative branches of the thallus.

As in other species of the Saprolegniaceae cleavage of the protoplasm in the sporangium and exit tube is progressive and mainly centrifugal in direction from the border of the vacuole, although peripheral furrows also may develop and progress centripetally. As soon as cleavage has been completed the central vacuole disappears as such, but the cleavage segments usually include an irregular vacuole as shown in FIGS. 1H and 39. Even so the sporangium and exit tube appear to be completely but loosely filled with zoospore initials (FIG. 1D). Particularly noteworthy is the occurrence of two or more rows or layers of zoospores in exceptional, large and broad sporangia. Cleavage may be unequal also with the result that unusually large segments are delimited. The

sporangium and its contents may persist in the stage shown in FIG. 1D for more than an hour, but eventually the tip of the exit tube deliquesces and the zoospore initials glide out in rapid succession. A large sporangium may be emptied in 30 to 45 seconds. As the zoospore initials glide out they become rod-like or cylindrical in shape to conform with that of the exit tube (FIG. 39). As each successive initial escapes from the orifice it squirms and pushes about to attain space among those which have already emerged. However, they do not exhibit any flagellar motility but round up in a cluster within 20 to 40 seconds after emerging (FIG. 1E). Accordingly, their initial behavior is like that of the zoospore initials of *Achlya* and *Aphanomyces*. At this stage the cluster of cystospores is loosely held together, as evidenced by the fact that they may be separated readily or sucked apart by rotifers feeding nearby. Later they adhere tightly together and cannot be separated by microdissection needles without injury. Furthermore, the cystospores in the interior of the cluster become hexagonal in median outline from mutual contact and pressure. Fairly often a few cleavage segments may remain in the sporangium and exit tube (FIG. E) where they degenerate or develop into sporelings (FIG. 1F). The number of cystospores formed by the sporangium depends on the latter's size. As few as 8 and as many as 160 have been counted in a cluster.

Individual cystospores, when free, are spherical in shape (FIG. 3) and vary from 6.8 to 10.2 μ in diameter. Exceptionally large ones derived from unusual cleavage segments may be up to 15 μ in diameter. Their protoplasm has the same structure and appearance as that of the sporangium, and usually an irregular vacuole is present in the center (FIG. 2).

Within 2 to 5 hours after encysting at the mouth of the exit tube, the cystospores germinate. However, in one unusual case observed they remained dormant for 42 hours before beginning to germinate, but in this instance the rudimentary zoospore degenerated before becoming actively motile. The process of germination (FIGS. 4–7) is fundamentally similar to that of the cystospores of *Achlya* and *Aphanomyces* and need not be described in detail. Immediately after emerging from the cyst the zoospore rudiment rounds up or it may become somewhat elongate (FIG. 7) or

lemon-shaped. Very shortly thereafter the place where the flagella are to develop becomes evident as a relatively hyaline area (FIGS. 8, 9), and the zoospore rudiment soon becomes subhemispherical in shape (FIG. 9). The flagella develop laterally or in the center of the so-called ventral groove and begin to beat slowly. This beating causes the zoospore body to rock back and forth, a motion which may continue from 20 to 40 minutes. At the end of this period the flagella suddenly begin to beat more rapidly and the zoospore swims away.

The mature zoospores are laterally biflagellate, elongately reniform, 6–6.7 × 9–10.4 μ, with a ventral groove and coarsely granular protoplasm (FIGS. 10–12). Occasionally, large 4- or 8-flagellate zoospores (FIG. 13) are formed from exceptionally large cystospores. The zoospores may swim actively for 7 to 12 minutes and in so doing they regularly turn over or rotate on their axis. Fairly often, however, they may swim but a short distance and come to rest on the substratum nearby the sporangium where they round up and later develop into sporelings. Accordingly, the zoospores of *S. spinosa* are monoplanetic as reported by Arnaudow, but they have a slight tendency towards repeated emergence. In eight instances zoospores were observed to form secondary cystospores which later germinated (FIG. 15), but the emerged protoplasts did not develop into zoospores. They merely settled down on the substratum and eventually developed into sporelings.

Sometimes zoospore formation may be suppressed altogether, and the primary cystospores at the orifice of the exit tube develop directly into sporelings. More frequently, a few of the cystospores become sporelings while the majority of them give rise to zoospores (FIG. 1F). Also, cleavage segments which failed to emerge from the sporangium (FIG. 1E) may develop into sporelings without apparently going through the cystospore stage (FIG. 1F).

Sommerstorffia spinosa reproduces also by the production of parthenogenic oospores, according to Arnaudow. The oogonia develop on special septate branches and contain a single egg. No antheridia were observed by Arnaudow, who consequently reported that the egg develops without fertilization. No oogonia and oospores have been observed in the Alaskan material in the course of more than a year. Inasmuch as Arnaudow's Bulgarian

paper may not be available to most American mycologists, I am presenting his one illustration of oogonia and oospores in FIG. 59.

HOSTS

As related at the start of this paper, *S. spinosa* is a parasite of rotifers, and Arnaudow and Sparrow reported it on a species of the genus *Monostyla*. Limited attempts were made in this study to secure additional information on its host range. What were advertised as cultures of *Monostyla, Euchlanis, Habrotrocha* and *Philodina* were bought from the Carolina Biological Supply Company, Elon College, N. C., grown in the manner described earlier, and used as hosts, but no attempt was made to verify the taxonomy of the genera and species. In addition, species of *Colurus* Ehrbg. and *Distyla* were isolated from pond water and tested as hosts. Of these six genera, *Monostyla, Distyla* and *Colurus* were captured in large numbers and devoured by *S. spinosa*. Species of *Euchlanis, Habrotrocha* and *Philodina*, on the other hand, were never captured. The species of *Habrotrocha* and *Philodina* used in this study were very large, fed voraciously on onion skin, and multiplied very rapidly so that they were present in great numbers. It is estimated conservatively that in the course of a year and a half more than a hundred thousand individuals of these two genera were observed feeding among the sporelings and mature thalli of *S. spinosa*, but none of them were found to be captured. At first it was thought that this might be due to their large size and ability to free themselves, but no evidence was found to support this view. Never were they observed to be temporarily caught on the predaceous pegs and sporeling. In fact, their ciliated corona was frequently observed in direct contact with the predaceous organs of the fungus. The fact that captured *Distyla* specimens were often as large as small individuals of *Philodina* and *Habrotrocha* is further evidence that size, within certain limits, is not a decisive factor. In mixed cultures of these six genera to which *S. spinosa* was added, the *Monostyla, Distyla* and *Colurus* individuals were completely depleted in the course of a week, while individuals of the other genera were unmolested.

These observations suggest offhand that *S. spinosa* may be somewhat specific in its hosts relations, but this does not seem

very plausible in light of the evidence that it captures protozoa as well as rotifers. The fact that some genera of rotifers are captured and others are not may be due to differences in structure of the mouth and feeding habits of the rotifers instead of to host specificity. Obviously, additional and more intensive studies with accurately identified host species are necessary to determine the host range of *S. spinosa*.

Among the Gastrotrichia, species of *Chaetonotus* were very abundant in our cultures, but none of these were captured by the fungus. Also present were numerous Infusoria of the genera *Coleps, Paramoecium, Stylonychia* and *Euplotes* which were never found captive. In numerous instances *Stylonichia notophora* was observed to engulf large numbers of newly-emerged cystospores without suffering any subsequent injury.

Of the flagellate protozoa or Mastigophora present in our cultures only *Entosiphon ovatum* was captured, although species of *Euglena* and *Peranema* were very abundant also. *Amoeba proteus* of the Amoebina was very common in two cultures and frequently enveloped the predaceous pegs and necks of the sporeling without being caught.

MECHANISM OF CAPTURE

Very little exact information is available on how *S. spinosa* captures its prey. Obviously, the predaceous pegs and the necks of sporelings are structurally adapted for this purpose, but quite probably it is the refractive protoplasm in them which is the more active agent. At least this is suggested by the occurrence of refringent or optically differentiated protoplasm in the spores, mycelium and specialized structures of other fungi such as *Zoophagus, Protascus* Dangeard, various members of the Zoopagaceae, and the Hyphomycetes which capture microscopic animals. Possibly, as the tips of the predaceous organs are "chewed" by the rapidly moving jaws of the rotifer they rupture, and the exuded protoplasm acts as an adhesive or mucilage. It should be stated here, however, that actual exudation of protoplasm has not been observed. On the other hand, it is quite possible that the predaceous organs may exude an adhesive substance without being ruptured, as Dreschler (**1937**) reported for numerous predaceous Hyphomycetes. In

these and retiary species he reported that the struggles of the captured animals stimulate the production of adhesive material and that as a result the latter becomes visible as a sizeable deposit. Attention is directed again to the tapering masses of hyaline material (FIGS. 46A–46E) on the tips of the predaceous pegs of *S. spinosa*. These might be equivalent to the deposits of adhesive material described by Dreschler. That the active agent may be an adhesive is further suggested by the observation that shortly or immediately after the rotifer gets caught its jaws cease moving and clamp shut as if they were stuck together. Possibly, this might be due to narcotic or poisonous effects, but this does not seem likely. If such effects occur they are very local because the cilia of the rotifer continue to beat unabated for a long time, and the rotifer uses its foot or feet vigorously for hours in turning, twisting and jerking to get loose.

The fact that *S. spinosa* can capture protozoa-like *Entosiphon ovatum* without jaws or chewing organs suggests that puncture of or injury to the predaceous pegs may not be necessary for capture. In numerous individuals observed the protozoa merely appeared to be stuck to the tip of the sporelings. It should be noted, nonetheless, that *E. ovatum* has a very long conical cytopharynx which is protrusible, and its action during feeding might possibly injure the predaceous peg.

The behavior of newly captured rotifers is characteristic. As related above the jaws cease to move almost immediately after capture, but the cilia continue to beat for two hours or more. At the same time the rotifers twist, turn and jerk with the aid of their foot or feet in a frantic effort to get loose. They may also contract periodically into their shells. These movements gradually become slower and weaker in the course of two hours or more and eventually cease. Then may follow a period of weak convulsive contractions after which the rotifer dies. Fairly often death may occur in the contracted position.

RELATIONSHIPS OF S. SPINOSA

The structure, method of formation, and behavior of the zoospores show clearly that *S. spinosa* is a valid member of the family Saprolegniaceae. Arnaudow regarded it as closely related to

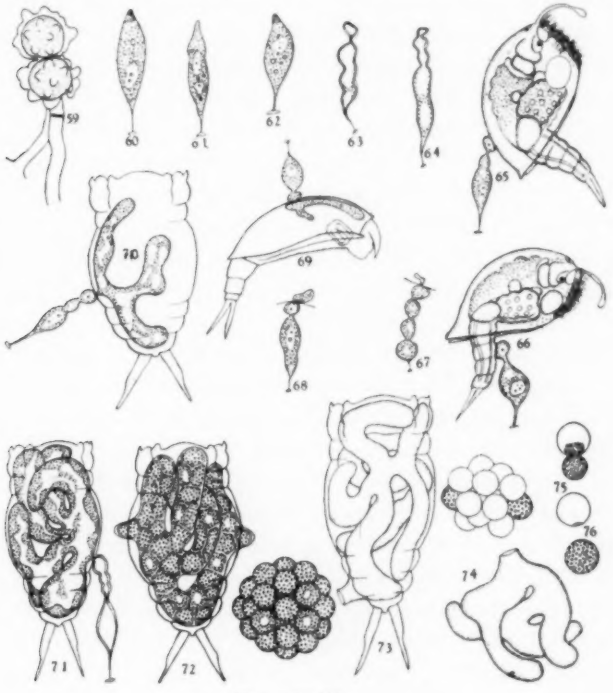
Aphanomyces and placed it next to this genus on the grounds that it does not have a well differentiated sporangium, its zoospores are formed in a single row, and that the oogonium contains but one oospore. On the basis of his observations, Valkanov (**1931, 1932**), Sparrow (**1942**) and Bessey (**1950**) followed Arnaudow's disposition of *Sommerstorffia* in the Saprolegniaceae. Until more is known about the development of its oogonia and oospores, this is probably the best disposition that can be made at the present time.

Nevertheless, it should be emphasized in this connection that the sporangia of *S. spinosa* are not always undifferentiated and slender as in *Aphanomyces*. Nor are the zoospore initials always formed in a single row. Most of the sporangia figured by Arnaudow are comparatively slender, but sometimes in our material they were quite broad, deeply lobed and even sub-globular (FIGS. 1B, 1C, 1D, 1E, 1F, and 39). As noted previously they may be up to 20 μ in greatest diameter and appear sharply differentiated from the more slender, tapering exit tube. Furthermore, in such sporangia the zoospore may be arranged in two, three and rarely four rows or layers as shown in FIGS. 1D and 39. Obviously the sporangium of *S. spinosa* varies considerably, and the arrangement of the zoospore initials may be similar to that of both *Achlya* and *Aphanomyces*. However, production of new sporangia by cymose branching as in *Achlya* has not been observed in *Sommerstorffia*, nor is the sporangium delimited from the rest of the thallus by a septum.

During the course of this study of *S. spinosa* it was observed that a few individuals of *Colurus*, *Monostyla*, and *Distyla* were caught by the foot or feet and on various regions of the lorica by minute fusiform thalli. At first it was thought that these thalli were modified sporelings of *S. spinosa* which are able to capture rotifers by contact and without being taken into the mouth of the host. Subsequent studies, however, revealed that they differ in structure and method of prey as well as in manner of subsequent development. Although the full life cycle of such thalli has not been observed because of limited material available, the data at hand suggest that they may relate to a new minute species of *Sommerstorffia*. On the other hand, such thalli have never been found in cultures in which *S. spinosa* was absent. Therefore, it

is difficult to avoid the thought that they relate in some unknown way to the life cycle of this species. Nevertheless, it seems worth while to present the known data, incomplete as they may be and regardless of whether they relate to *S. spinosa* or a new species.

The thalli mentioned above are usually broadly or narrowly fusiform in shape (FIGS. 60–62), stalked, and vary from 15 to 24 μ



FIGS. 59–76.

FIGS. 59–76. 59. Oospores of *S. spinosa*, after Arnaudow (**1923b**). 60–76. *Sommerstorffia* sp. (?). 60–62. Stalked fusiform thalli with coarsely granular vacuolate protoplasm. 63, 64. Irregularly-shaped thalli which may have been attached to rotifers. 65, 66. Thalli attached to dorsal surface and foot, respectively, of *Colurus* sp. 67–69. Early stages of development of endobiotic portion of thallus. 70. Later stage of development; content of rotifer body omitted. 71. Mature endobiotic thallus with coarsely granular, vacuolate protoplasm; note thick walls of empty epibiotic thallus. 72. Endobiotic sporangium with cleavage segments; epibiotic thallus has disappeared. 73. Cystospore cluster at orifice of exit papilla. 74. Empty endobiotic sporangium and cystospores. 75, 76. Germination stages of cystosphores.

in overall length by 5 to 10 μ in greatest diameter. Usually, the stalk is filamentous, 4 to 11 μ long, and subtended by a small, 2.5–3.6 μ diam., oval or circular, flattened foot or holdfast, which is strongly adherent to or imbedded in the substratum. The protoplasm in these thalli usually is coarsely granular with one or more small vacuoles which contain minute dancing granules. The content of the tapered apex, on the other hand, is more optically homogeneous and refractive than the remainder of the protoplasm, but it does not appear to be as sharply differentiated or as refringent as the substance in the predaceous pegs of *S. spinosa*. Generally, one or several refractive globules are present in the granular protoplasm (FIGS. 60–62). So far we have not observed a separation of the protoplasm into refractive apical and basal portions as in the sporelings of *S. spinosa*. This, together with their shape, presence of a stalk and foot or holdfast, is the principal structural difference observed so far between these thalli and the sporelings of *S. spinosa*.

Like the latter they hang down from the under surface of the substratum and, as stated earlier, are capable of adhering to the shell, foot or any other part of the rotifer body. FIGS. 65 and 66 show thalli attached to the dorsal surface and foot, respectively, of *Colurus* sp., and FIGS. 70 and 71 show similar thalli attached laterally to *Distyla* sp. The method by which they become attached is not known. Presumably, it occurs by adhesion because no specialized structural organs for capturing prey are present, as far as we know. Actual capture has not been observed. In a few instances rotifers were seen to come into contact with or rub against the apices of thalli without being caught, which indicates that brief contact does not always result in capture. Possibly, frequent or continuous contact and irritation by the rotifer for a longer period might stimulate the secretion of an adhesive substance by the apex, as described by Dreschler for other predaceous fungi, but in light of the lack of direct observational evidence this view is purely speculative. All that we have observed so far were captive rotifers strenuously trying to pull away and free themselves. Quite probably, some of them get away but we have not seen them do so. A few contorted thalli with swollen apices (FIGS. 63, 64) have been found which resemble those attached to rotifers,

which suggests that the latter succeeded in escaping. Those which are caught permanently struggle desperately with all the means at their disposal to get loose. In doing so, they push against the substratum with their feet or foot, lash and twist back and forth, and at the same time spin about in the water. During such spinning movements the apex of the thallus may be twisted around in several complete turns, and it is remarkable that the apex is not broken off. In such instances it seems to operate like a universal joint. The twisting and spinning action of the rotifer might possibly be the cause of the catenoidal structure of some of the attached thalli (FIG. 67). In 1½ to 3 hours the rotifer ceases to struggle and eventually dies. Death appears to be due partly to exhaustion.

Although we have not observed the process, the apex of the thallus apparently enlarges and becomes globular after attachment to the rotifer. Usually one side of the enlargement is closely flattened against the host (FIGS. 65–67) and has the appearance of an appressorium. Probably, it functions as such. From this structure develops a very narrow germ tube or filament which penetrates the shell of the rotifer and then swells into an elongate vesicle (FIGS. 67, 68). This vesicle continues to elongate into a tubular structure inside and closely adjacent to the shell (FIG. 69). With further growth it branches (FIG. 70) or becomes deeply lobed, increases in diameter, and eventually fills the body of the host (FIG. 71). The development of the endobiotic thallus is, thus, identical with that of *S. spinosa*. Furthermore, the protoplasm has the same appearance and structure, being very vacuolate and coarsely granular (FIGS. 70–72).

No further development of the small epibiotic thallus occurs, as far as we know. Shortly after the stage shown in FIG. 71, it disappears or is broken off by the movement and feeding of other rotifers in the culture. This lack of epibiotic growth and development of the external thallus is another difference which seems to distinguish it from the sporelings of *S. spinosa*. The mature endobiotic thallus is transformed holocarpically into a zoosporangium, and its protoplasm undergoes cleavage into zoospore initials (FIG. 72) in the same manner described previously for *S. spinosa*. Before cleavage, however, 1 or 3 low, broad, tapered, 8–11 × 12–14 μ, exit papillae are formed (FIG. 72), and these appear to project

directly through the rotifer shell. In no instances were long exit tubes observed like those of *S. spinosa*. When the tip of the exit papilla deliquesces, the zoospore initials glide out quickly in succession and encyst in a cluster (FIG. 73) near the orifice without exhibiting any flagellar movements. The cystospores may persist for 2 to 8 hours and then germinate (FIGS. 75–76) as in *S. spinosa*. No fundamental differences in this process as well as the development of the ventral groove and flagella have been observed.

Unfortunately, at the time these stages of zoosporogenesis were found it was assumed that they related to *S. spinosa*. Consequently, no measurements were made of the cystospores and zoospores. Nevertheless, it should be mentioned that they seemed strikingly similar in size, shape and behavior to those of *S. spinosa*. Germination of the zoospores and their development into thalli were not observed.

Accordingly, there are many gaps in our knowledge of the life history of this fungus. Until these gaps have been filled it is not certain, therefore, whether our observations relate to a new species of *Sommerstorffia* or to an unusual developmental phase of *S. spinosa*. Nevertheless, the difference in structure of the minute thalli, their lack of specialized predaceous organs and ability to capture rotifers on any part of the body as well as the absence of epibiotic development suggest at least that we may be dealing with a new species.

SUMMARY

Sommerstorffia spinosa, a predaceous species of the family Saprolegniaceae which captures and devours rotifers, was collected in August, 1950, in soil from an alpine tundra stream at an altitude of 6500 feet in the Talkeetna Mountains in Alaska. This is the third published report of its occurrence in nature. It consists of 1 to 6 or rarely more, straight or slightly curved, 60–200 μ long by 5.6–10.4 μ broad, comparatively rigid, non-septate, tubular branches which are terminated by a specialized, narrow tapering peg or blunt spine filled with homogeneous refractive substance. These pegs presumably secrete an adhesive substance when taken into the mouth of the rotifer, which is then unable to free itself.

The fungus invades and absorbs the internal organs of its host, and usually forms an endobiotic sporangium whose content undergoes cleavage into zoospore initials. The latter emerge singly in succession without flagellar action from long exit tubes and encyst nearby in clusters, and later germinate to form elongately reniform, 6–6.9 × 9–10.4 μ, laterally biflagellate zoospores. These eventually come to rest on the underside of a suitable floating substratum and develop into flask-shaped, 6.8–12 × 13.6–20.4 μ, sporelings which adhere strongly to the substratum and hang down in the water. Their necks are filled with a refractive substance and are as capable of capturing rotifers as the predaceous pegs of the mature thallus.

Sporelings which have captured rotifers usually develop into a dichotomously branched epibiotic thallus and an endobiotic sporangium. Occasionally, they give rise to a completely epibiotic thallus or only an endobiotic sporangium. Oospore development was not observed in the Alaskan material.

Species of *Monostyla, Distyla, Colurus* were readily captured in large numbers by *S. spinosa,* but members of *Euchlanis, Habrotrocha* and *Philodina* were never found captive. *Entosiphon ovatum,* a protozoan, was frequently caught by the sporelings and predaceous pegs of the mature thallus.

Thalli of what may prove to be another species of *Sommerstorffia* were found in the Alaskan material. These thalli are broadly or narrowly fusiform, stalked, vary from 15 to 24 μ in overall length by 5 to 10 μ in greatest diameter and are subtended by a small oval or circular, 2.5–3.6 μ diam., flattened foot or holdfast, which is strongly adherent to or embedded in the substratum. They lack structurally specialized organs of prey, and apparently are capable of capturing rotifers by contact with any part of the body. They give rise to an endobiotic sporangium which forms zoospore initials, cystospores, and zoospores as in *S. spinosa.* No epibiotic development of thalli was observed.

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INTERFERTILITY BETWEEN TWO DISTINCT FORMS OF CYATHUS OLLA

HAROLD J. BRODIE

(WITH 8 FIGURES)

One of the largest and most striking of the bird's nest fungi that has come to the writer's attention is one collected by Mr. Lindley D. Carson near Corbett, Oregon, November 5, 1950. The large peridioles and the spore size both suggested some relationship of the Oregon fungus to *Cyathus olla* Pers. However, a glance at FIGS. 1 and 2 will show that, superficially at least, the difference between the two fungi is great.

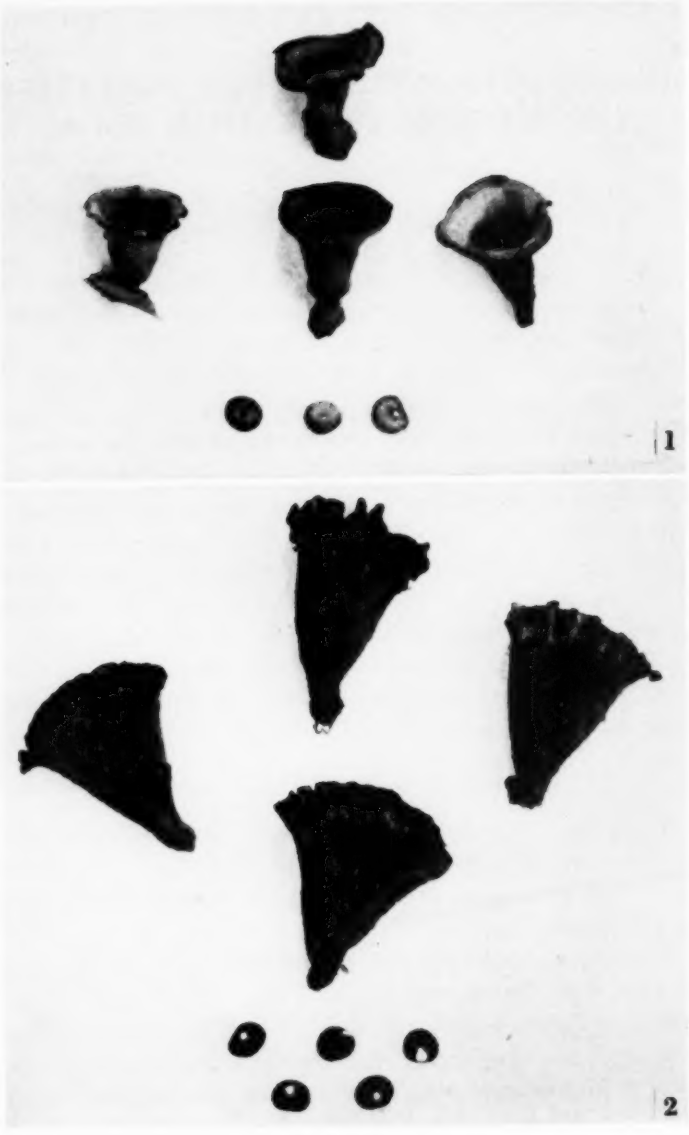
As Mr. Carson's specimens had been sent by air mail, spores were found to be viable and cultures were made at once. The present paper reports the results of a study of the Oregon fungus in culture, an analysis of sexual behavior of single-spore mycelia, and successful hybridization between these mycelia and certain haploids of *C. olla*. In addition, a study has been made of the taxonomic status of the Oregon fungus which has been identified with *Cyathus olla* form *anglicus* Lloyd.

DESCRIPTION OF THE OREGON FUNGUS

Mr. Carson reported that the plants had been found growing on straw debris in an open pasture near Corbett, Oregon. About two dozen cups grew in an area six inches in diameter but no other specimens were found in the region.

Typical fruit-bodies are well represented by FIG. 2. The cups are dark umber brown, some almost black. They measure 15 mm. across the mouth and 18 mm. high. The shape is broadly campanulate. The mouths are only slightly reflexed and are markedly sulcate: in some specimens the mouths are very irregular in outline and might be described as dentate.

The exterior of the cup is covered with a dense tomentum of fine appressed hairs but the fruit bodies are not at all shaggy as



FIGS. 1-2.

are those of many species of *Cyathus*. The peridium wall as a whole is thick and rigid.

The base of the cup is more elongate than in most bird's nest fungi. Attached to this stipe-like portion is a heavy emplacement which, however, had become detached in the large specimens chosen for the photograph (FIG. 2).

On the inside, the cups are smooth, grey-brown, shiny, and inconspicuously longitudinally ridged.

The unusually large peridioles (3.5–4 mm.) are flat and irregular in outline. They are somewhat darker than those of *C. olla* but have the same kind of delicate tunica.

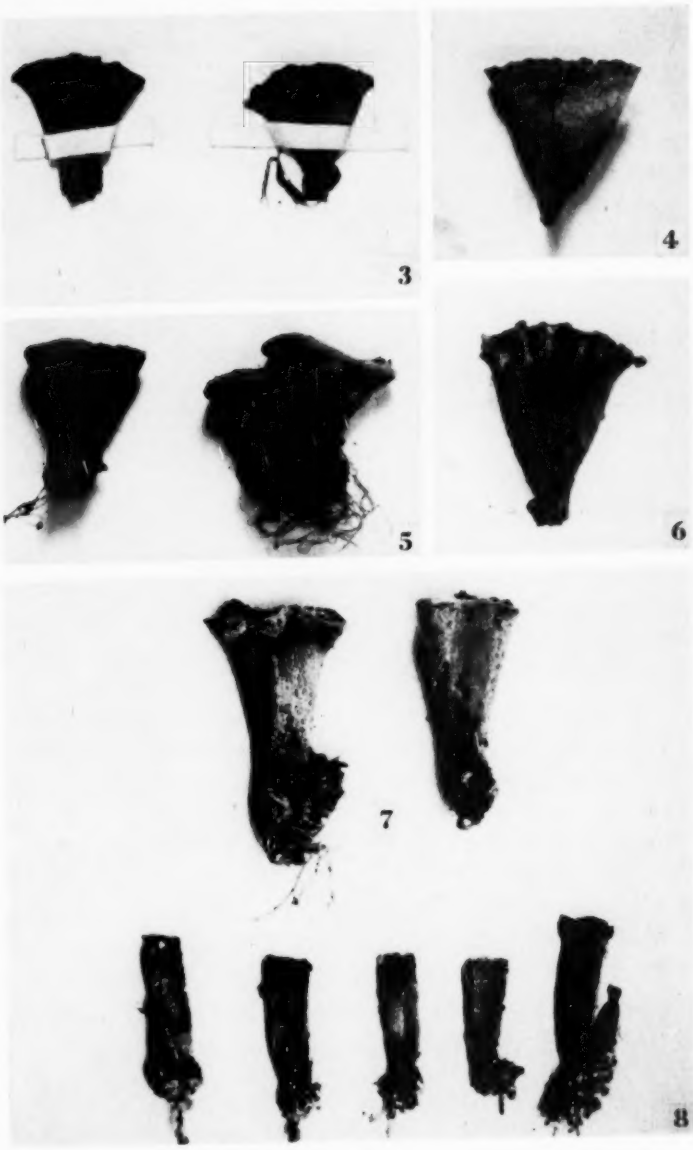
The basidiospores are ovate, have a thin wall and measure 7.5–9 × 11.5–12.5 μ.

LLOYD'S CYATHUS ANGLICUS

In his monograph of the Nidulariaceae, Lloyd (**4**) described a fungus from the Kew Herbarium to which he gave the name *C. anglicus*. This he considered to be a form of *C. olla* and he believed it to be solely English, though he stated: "The plant Miss White illustrates from Colorado under the name *Cyathus dura* seems to me very much the same nature."

Through the courtesy of Mr. J. A. Stevenson, the writer has examined the single type specimen of *C. anglicus* now in the Lloyd Collection in the Smithsonian Institution. It bears the label "*06598, C. anglicus* TYPE, Ex. Herb. Kew, England." Except for its pale color, Lloyd's type agrees well with the description of the Oregon fungus given above. The specimens shown in FIG. 2 of this paper are almost exact counterparts of the photograph of *C. anglicus* published by Lloyd (**4**, Fig. 17, p. 25). A photograph of Lloyd's specimen in its present condition is shown in FIG. 4 of this paper. Spores of the type measure 7.5 × 10.5–12.5 μ and the peridioles are large (3.5 mm.) and flat.

FIGS. 1–2. *Cyathus olla*, typical common form collected in Winnipeg, Canada. Spores from one of these fruit-bodies provided the mycelia described in this paper. × 1.5. 2. *Cyathus olla* form *anglicus* from Oregon. Spores from one of these fruit-bodies provided mycelia described in this paper. × 1.7.



FIGS. 3–8.

Miss E. M. Wakefield kindly sent to the writer for examination three collections selected from the Kew Herbarium for their agreement with Lloyd's conception of *C. anglicus*. Two proved very similar to Lloyd's type specimen. One of these (from Berkeley's herbarium), bearing the date stamp 1867, consisted of two specimens with large sulcate cups and prominent emplacements. The other (labeled "Charlton Kent, D. C."), bearing no date, was almost identical with Lloyd's type. The spores measured 6–8 × 11–12.5 μ. The latter specimens are shown in FIG. 3.

For comparison, two photographs of *C. olla* are reproduced. FIG. 1 shows cups collected by the writer in Winnipeg, Canada, September 7, 1941, and FIG. 5 some of the same species collected at Kew, July 18, 1950.

From the study of English material, including Lloyd's type, and other similar specimens from Kew, it is clear that the fungus called *C. anglicus* by Lloyd is a form which is readily distinguishable from the typical form of *C. olla* and that the fungus collected apparently for the first time in North America is also properly referred to *C. olla* form *anglicus*. In passing, it may be noted that *anglicus* is not the only form occurring in Oregon, for the writer has examined several collections of the typical form of *C. olla* from that state.

C. dura, described by Miss White (**5**) from Colorado, has not been studied but, to judge from the description, it is quite possible that *C. dura* is the same as *C. anglicus*.

One other collection deserves mention. Mr. Jorge Wright of Buenos Aires sent to the writer three fruit bodies collected in 1950 near that city. The specimens are very similar to some of *C.*

FIGS. 3–8. 3. *Cyathus olla* form *anglicus;* specimens from the Royal Herbarium, Kew, England, from Berkeley's collection. × 1.5. 4. *Cyathus olla* form *anglicus;* Lloyd's type specimen now in the Smithsonian Institution (Washington, D. C.), number *06598*. × 1.5. 5. *Cyathus olla*, typical specimens collected by H. J. Brodie, Kew, England, 1950. × 1.7. 6. *Cyathus olla* form *anglicus*. One of the specimens illustrated in FIG. 2, placed here for comparison. × 1.7. 7. Fertile fruit-bodies developed from mating of an *AB* haploid mycelium of *Cyathus olla* and an aB^1 haploid mycelium of *C. olla* form *anglicus*. Note light color, elongate form and sinuous mouth. × 1.9. 8. Sterile fruit-bodies developed from mating of an *ab* haploid mycelium of *Cyathus olla* and an Ab^1 haploid mycelium of *C. olla* form *anglicus*. Note dark color and elongate form. × 1.9.

anglicus from Kew and only slightly smaller and paler than the Oregon material.

It thus appears that *C. olla* form *anglicus* is not confined to England as was supposed by Lloyd, but that it occurs also in North America and possibly in South America.

CYATHUS OLLA FORM ANGLICUS IN CULTURE

It has been shown by the writer (**2**) that *Cyathus olla* is heterothallic and that its haploid mycelia fall into four mating types when paired in all possible combinations. It seemed desirable to analyze the sexuality pattern in the form *anglicus* for purposes of comparison.

Basidiospores suspended in distilled water required four days to germinate at 40° C. This is a longer germination period than any heretofore observed for a member of the Nidulariaceae. During germination, basidiospores swell slightly and evenly. Each spore produces one stout germ tube only which emerges from the small end of the spore and which becomes quite long before branching. Haploid mycelia are fluffy, fine-textured and white or light buff colored.

On the basis of their pairing reactions, the haploid mycelia were assigned to four mating types as shown in TABLE I.

TABLE I

DISTRIBUTION OF FOURTEEN HAPLOID MYCELIA OF *Cyathus olla* FORM *anglicus* WITH REGARD TO MATING TYPE

Mating type	Culture number
AB	2, 9, 12, 16
ab	4, 5, 6, 14, 15
Ab	3, 8, 13
aB	7, 10

From these results it appears that *C. olla* form *anglicus* is like all other members of the Nidulariaceae studied up to the present time in being heterothallic and tetrapolar.

PAIRINGS BETWEEN HAPLOID MYCELIA OF CYATHUS OLLA AND OF THE FORM ANGLICUS

Haploid mycelia of known genotype of the typical form of *C. olla* had been kept alive since the writer's study of that fungus in 1949

(2). Eight of these were selected from among the most vigorous of the cultures that had survived, namely:

AB: Nos. 1, 4
ab: Nos. 2, 14
Ab: Nos. 3, 11
aB: Nos. 12, 18

These cultures had been isolated November 19, 1948, from a fruit-body originating in Winnipeg, Canada.

The eight haploids of *C. olla* were then paired in all possible combinations with eight haploids of *C. olla* form *anglicus*. For convenience, in the following table, cultures of *C. olla* are referred to as O_1, O_4, etc., those of *C. olla* form *anglicus* as F_2, F_9, etc. TABLE II gives the results of the pairings, where a + sign indicates the development of binucleate mycelium bearing clamp connections.

TABLE II

RESULTS OF PAIRING HAPLOID MYCELIA OF *Cyathus olla* (O_1 ETC.) WITH HAPLOID MYCELIA OF *C. olla* FORM *anglicus* (F_2 ETC.)

	F_2	F_9	F_4	F_5	F_3	F_8	F_7	F_{10}
$O_1(AB)$			+	+			+	+
$O_4(AB)$			+	+			+	+
$O_2(ab)$	+	+			+	+		
$O_{14}(ab)$	+	+			+	+		
$O_3(Ab)$			+	+			+	+
$O_{11}(Ab)$			+	+			+	+
$O_{12}(aB)$	+	+			+	+		
$O_{18}(aB)$	+	+			+	+		

In TABLE II, we see that F_4 and F_5 are sexually compatible with O_1 and $O_4(AB)$ and with O_3 and $O_{11}(Ab)$. Since only those mycelia that give completely heterozygous combinations are sexually compatible, F_4 and F_5 cannot possess the genes *A*, *B*, or *b*. They could possess the gene *a* and either of a pair of alleles B^1 or b^1; i.e., they could have the constitution aB^1 or ab^1.

If F_4 and F_5 possessed any gene other than *a* (e.g., a^1 or A^1) and the genotypes were a^1B^1 or a^1b^1, then F_4 and F_5 would be

compatible with O_2 and O_{14} and with O_{12} and O_{18} since any of these combinations would result in complete heterozygosity and consequent sexual compatibility. Therefore, F_4 and F_5 must be aB^1 or ab^1.

By similar reasoning, F_2 and F_9 must be AB^1 or Ab^1. Since (from TABLE I) we see that F_2 and F_9 are incompatible with F_3, F_8, F_7 and F_{10}, and (from TABLE I) that F_3 and F_8 are compatible with F_7 and F_{10}, it is possible to conclude that the following formulae should be applied to the eight mycelia of *C. olla* form *anglicus:*

AB^1: 2, 9
ab^1: 4, 5
Ab^1: 3, 8
aB^1: 7, 10

These results show that *C. olla* and *C. anglicus* are but different forms of the same species since haploid mycelia of the two forms are sexually compatible in certain combinations. The nature of the interaction of the two forms shows that, of the two pairs of genes governing sexuality, *C. olla* and its form *anglicus* both possess one gene pair in common (*A* and *a*) whereas the two fungi differ in the other pair of genes, which are designated as *B* and *b* for the former fungus and B^1 and b^1 for the latter.

Nils Fries (**3**) has already shown that allelomorphs for heterothallism in the Nidulariaceae are fewer in number than in the Agaricales. The recognition of one pair of identical genes in *C. olla* and form *anglicus* is therefore in line with Fries' findings.

FRUITING OF HYBRID MYCELIA

The hybrid mycelia referred to above showed no sign of fruiting on agar medium. A number of them were kept growing on straw moistened with the nutrient solution used previously by the writer (see Brodie, **2**). When these cultures were a month old they were transferred to six-inch bulb pans, the mycelia being covered with an inch of sterile loamy soil. The pots were then stood on the greenhouse bench in shade.

Rudiments of fruit-bodies were visible on the soil in several pots seven days after the mycelia had been covered with soil. The fruit-

body rudiments in most of the cultures did not develop further and soon died.

In two cultures mature fruit-bodies had developed by the end of the third week after the mycelia had been planted in soil. These will now be described separately. It should be noted that cultures of *C. olla* were fruited on the same medium at the same time for purposes of comparison with the hybrids. Cultures of *C. olla* form *anglicus* did not fruit.

Culture O_2F_3 (*olla ab* × *anglicus Ab*^{1}). The hybrid fruit-bodies (FIG. 8) are dark brown and curiously cylindrical. They are 10–13 mm. high and 4 mm. wide. The mouths of most specimens are slightly sinuate or dentate like those of *C. olla* form *anglicus*. Although thirty-four fruit-bodies all very much alike were produced on the culture, not one of them contained a mature peridiole. A few abortive peridioles were developed in two specimens but no spores were found in them.

Culture O_4F_7 (*olla AB* × *anglicus aB*^{1}). Only two mature fruit-bodies (FIG. 7) developed on this culture. They are pale grey in color like the cups of the typical form of *C. olla*. The fruit-bodies differ from either parent in being larger. They are 20 mm. high and 9–13 mm. across the mouth. The columnar form of both specimens is distinctive. The mouths are only slightly sulcate.

Both specimens produced peridioles within which spores were developed. The spores measured 8–9 × 11–12 μ. These spores have a normal appearance and are probably viable though attempts to induce their germination have not been successful as yet.

DISCUSSION

The differences in size, form and color between *Cyathus olla* and the form *anglicus* are actually much greater than the differences between many so-called "good" species of the Nidulariaceae and it reflects credit on Lloyd's judgment that he refrained from describing *anglicus* as a new species but retained it as a form of *C. olla*. Lloyd's position seemed rational because it is becoming increasingly apparent that much variation exists within many of the common members of the Nidulariaceae. For example, the writer (1) has shown that in *Cyathus stercoreus* (Schw.) De Toni,

it is possible to obtain many different types of fruit-bodies in culture all originating from spores of one wild-type peridiole.

Study of *C. olla* and form *anglicus* in culture confirms the relationship between them suggested by Lloyd. Although no detail has been reported above, it may be noted simply that the two fungi are not distinguishable in the mycelial stage: the mode of germination of basidiospores is the same and the appearance of haploid and diploid mycelia the same in both forms.

The interfertility of the two forms (in some pairings) does not give final proof of their species identity, since it is possible for two species of higher basidiomycetes to hybridize. However, such interspecific crosses have been attempted frequently and usually without success. If *olla* and *anglicus* were different species capable of hybridizing, one might expect them to be either completely interfertile or partially interfertile with no regular pattern to the interfertility. The fact that the haploid mycelia of the two fungi under discussion form dikaryotic mycelia only in combinations which demonstrate that they possess one pair of sexuality genes in common and differ in the other pair, argues strongly for the conclusion that the two fungi are forms of one species.

Of the two types of hybrid fruit-bodies developed in culture, neither is exactly like either parent form. The O_2F_3 fruit-bodies are dark in color and have sinuate mouths like the *anglicus* parent. The columnar form of these hybrids is a character not seen in either parent. Since the fruit-bodies are all sterile no further study of them is possible.

The fruit-bodies of the culture O_4F_7 are like typical *C. olla* in color but they have the large size and sulcate mouths of form *anglicus* and, in addition, a columnar shape which is not characteristic of either parent. To date, several attempts to induce germination of the spores of these hybrid fungi have been unsuccessful but further effort is being made.

Study of the progeny of the hybrids is necessary as proof of hybridity. However, since the experiments recorded above were begun using haploid mycelia of the two distinct forms which when paired produced diploid mycelia, one has either to assume that the diploid mycelium was formed as a result of the union of the two haploids or that it arose as a result of a mutation of either haploid

to diploid. The latter would be a rare occurrence and its probability is reduced by the regular pattern of compatibility of the paired mycelia shown in TABLE II and still further reduced by the difference between the hybrid fruit-bodies and those of either parent fungus.

SUMMARY

1. A large and distinctive *Cyathus* from Oregon has been identified as *C. olla* form *anglicus* described by Lloyd from a specimen from the Kew Herbarium, England.

2. The known distribution of *anglicus* includes England, Oregon (U. S. A.), probably Colorado (U. S. A.), and Buenos Aires (Argentina).

3. *C. olla* form *anglicus* is very like *C. olla* in the manner of germination of spores, the appearance of haploid and diploid mycelia, and in being heterothallic and tetrapolar.

4. Certain haploid mycelia of *anglicus* produce diploid mycelia when paired with certain haploids of *olla*.

5. The interaction of the mycelia of the two forms shows that they both possess the *A* and *a* pair of sexuality genes and that the two forms differ in the other pair of sexuality genes.

6. Two kinds of hybrid fruit-bodies were obtained in soil cultures from two different diploid hybrid mycelia. Neither type of hybrid fruit-body was exactly like either parental fungus.

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REVIEWS

THE AGARICALES IN MODERN TAXONOMY, by Rolf Singer. Lilloa **22**: 1–832. 24 pls. 1949 (appeared in August, 1951).

The appearance of this book, published as volume 22 of the journal Lilloa, has been anxiously awaited for a long time by those who have followed Dr. Singer's work on generic concepts among the agarics and boletes. It is dedicated to Vincent Fayod and Narcisse Patouillard, which is fitting, as it is a projection of the work of these men. There is a general introduction—a "critical survey of the characters of the Agaricales as the basis of their taxonomy," and a "special part"—a treatment of the families, genera and species of the Agaricales in systematic arrangement. It is well indexed, including an index to the technical terms used, with page references for each to aid in locating the discussions dealing with them.

In the critical survey one finds quite detailed presentations of gross characters such as veils and general fruiting-body morphology, as well as detailed discussions of anatomical features, spore characters, chemical characters etc. This chapter is essential to the use of the book and an understanding of the author's concept of the various characters. Since the aim is a natural classification, there are chapters on phylogeny also. The organization of the special part is very well done and makes the work easy to use. First, the order Agaricales is presented followed by a discussion of the various concepts which have been used and the author's concept. This is followed by a technical description of the order and then a key to families. The key is followed by a listing of the 15 families with their included genera. The families are then taken up in numerical order. The type genus is listed for each: there is included a technical description and a key to the genera. Each genus is then taken up according to the same pattern, but keys to species are included for only a few genera. Under the heading "Species" the author gives the subdivisions of the genus and cites those species which he is willing to accept. This is very

important. Not all the known species for each genus are included. By limiting himself to those fungi he had studied himself or which are unquestionably well known in the literature, the author has avoided many pitfalls. The important synonyms are given for many species.

The over-all aspects of the book are excellent. As the most critical analysis of generic concepts in the group to date, this book is a necessity for everyone doing any work on gill fungi. Dr. Singer has been studying his subject for years, and has collected fungi in many parts of the world (Austria, the Caucasus Mts., Spain, France, northern USSR near Leningrad, The Altai Mts. of Central Asia, North America, both in New England and Florida, and most recently in South America). This wealth of field experience coupled with the author's exceptional linguistic talents is amply reflected in his work. He has demonstrated his mastery of the literature as well as comprehensive knowledge from a world standpoint. I know of no one better qualified for the task he undertook.

The book deserves special comment in regard to nomenclatorial problems. Singer has made a serious effort to bring agaric nomenclature in line with the International Rules. For this we all owe him a sincere vote of thanks. He has been careful to list the species which he accepts as the type for each genus and the types of the sections of genera as well. It is to be hoped that starting from this work, investigators can at least disagree in an orderly manner on generic concepts without being plagued in addition by the old difficulty of the use of the same generic name—but typified differently—by various authors, each in a different sense. Since the matter of having the Congress act to conserve type species of the older genera (where none was originally designated) has not been too well received, and since it appears to be getting out of hand because of proposals by some who are not specialists in the group in which they propose lectotypes, it seems best to me now to simply accept the designations of type species for genera as made by Singer in his work. His publication meets all the requirements for such a work. 1. It represents a large amount of original research over many years. 2. It is based on careful evaluation of the literature. 3. It is more comprehensive than any other published revision.

4. It is more closely in line with the International Rules than any previous similar publication.

As stated previously, 15 families are recognized and are treated in the following order: 1. Hygrophoraceae, 2. Tricholomataceae, 3. Amanitaceae, 4. Agaricaceae, 5. Coprinaceae, 6. Bolbitiaceae, 7. Strophariaceae, 8. Cortinariaceae, 9. Crepidotaceae, 10. Rhodophyllaceae, 11. Paxillaceae, 12. Gomphidiaceae, 13. Boletaceae, 14. Strobilomycetaceae, and 15. Russulaceae. The author starts with the Hygrophoraceae, a group of uncertain origin, and progresses through the Tricholomataceae to other families showing more highly specialized anatomical features or fruiting body or more highly organized spores. The Russulaceae are, of course, regarded as a completely separate line. In the main this trend of evolution in the group appears sound, although I do not agree in all details. Certainly an analysis of the hyphal arrangements in the carpophore and the spore characters, both of which Singer has used, are about as fundamental a set of characters as we can hope to get in these fungi. In my estimation the problem of the true relationships of the Agaricales still awaits critical monographs of the individual genera, but it will be years before enough of these are available to bring much new information to bear on the over-all picture. In the interval Dr. Singer's work will fill a need for a summary and serve to guide monographers.

The book is written in English, and the author is to be congratulated on the clarity of his expression, and on the good editorial work, which adds greatly to the usefulness of the work. No detailed attempt will be made here to analyze his generic concepts. These will form the meat of much discussion in the future as the world flora of the Agaricales becomes better known. Dr. Singer cannot be accused of being either a "splitter" or a "lumper" (bad terms at their best), for in some groups he has erected genera on what to some will appear to be obscure differences whereas in others only one genus is recognized where many existed in the Friesian system. This is to his credit.

I personally cannot attribute the importance to the presence or absence of clamp connections which seems to be the modern tendency. To me it is a character to be used primarily at the species level. In *Cortinarius*, one genus in which clamps are supposed

to be constant, there are a few species which do not have them, yet I would never consider making this group a separate genus. I am also inclined to go slowly in placing importance on many of the new chemical characters (mostly color reactions). My own experience during the last 3 years with strong bases such as KOH in *Cortinarius* has shown them to be erratic in some groups. At the present time, if two species can be distinguished only by a color reaction to a certain chemical, I would not be inclined to recognize them as distinct at the species level. I would agree with Singer and other investigators that after one knows the species, some color reactions are handy for quick identification, especially of herbarium specimens, but this is quite a different matter from placing great emphasis on such characters in arriving at species concepts.

In the chapter on cystidia the author has confused a set of terms meant to apply only to position of the sterile element as contrasted with a system of names based in part on supposed function and in part on morphology. The terms pleurocystidia, cheilocystidia, pilocystidia and caulocystidia are to be used to indicate position, and as such serve a very important purpose in the description of agarics. In his classification based on type and function, I can't help but wish that he had been ruthless in throwing out long and cumbersome terms and arriving at a set of terms both convenient and appropriate. Taxonomists are not likely to take up "dermatopseudocystidia" and "dermatopseudoparaphyses" in favor of unrecommended but more convenient terms. In the chapter on spores, a listing of the types of ornamentation is very helpful, but one can't help but raise his eyebrows at "Type IX. Ornamentation continuous, a smooth surface resulting." As usual, Singer has placed great emphasis on the amyloid reaction (color in Melzer's solution). While I do not belittle the character, still I believe that it is being given more emphasis than it deserves, at least in *Mycena*. The term amyloid, as pointed out by Singer, has different meanings. Spore walls which in Melzer's solution are gray to violet-black are *amyloid*. Spore walls that become dark rusty to purple brown are *pseudoamyloid*. Those remaining hyaline or becoming yellowish are *nonamyloid*. In hyphae, if the walls become vinaceous brown, as in part of *Mycena*, they are called

amyloid. If the hyphal walls become violet as in some species of *Lentinus* sensu lato and in *Hericium* they are also called *amyloid*. It would have been relatively easy to have applied these terms in a consistent manner, even if this had not been done previously.

In summary, it should be said that Dr. Singer is to be congratulated on a fine piece of work which is certain to place him as the foremost world authority on the subject of generic concepts in the Agaricales. No one, I am sure, realizes more than Dr. Singer the extent of the work yet to be done. As he predicts, when we really have accurate data on sufficient species, we will find that the distribution of agarics over the world's land masses has more meaning than many have heretofore supposed.—ALEXANDER H. SMITH.

KLÍČ KURČOVÁNÍ NAŠICH HUB HŘIBOVITÝCH A BEDLOVITÝCH (AGARICALIUM EUROPAEORUM CLAVIS DICHOTOMICA), by A. Pilat. 722 pp., including 240 pp. of photographs, 2 line drawings. Quarto. Brazda, Prague. 1951. Price, $16.00 (approx.).

The author, well known for his many previous works on the fungi of Europe and of Czechoslovakia in particular, presents a large quarto volume on the Agaricales of Europe, considering the group in the modern sense as including the Boletaceae and excluding the Cantharellaceae. Other families recognized are Gomphidiaceae, Paxillaceae, Hygrophoraceae, Russulaceae, and Agaricaceae, the latter with fifteen subfamilies. The book is much broader in its coverage than the title would indicate since, although the material is presented in the form of detailed dichotomous keys to families, subfamilies, genera and species, other features are added including extensive literature citations for the order in general, and for each minor subdivision down to and including the species. Each species as it is keyed out is described technically, with synonomy and literature citations. 740 photographs comprising nearly half the volume are excellent. In addition a glossary of Czech and Latin terms is a useful feature and the indexes to Czech common names and to Latin generic and specific names comprehensive. Many will regret that the text is in Czech, but those concerned with this group of fungi will readily contrive to make good use of a

compendium of knowledge brought together by a master in the field.—JOHN A. STEVENSON.

PHYTOPATHOLOGIE DES PAYS CHAUDS (Encyclopedie Mycologique, vol. XVII), by L. Roger. Vol. I. xvi + 1126 pp., 153 figs. Paul Lechevalier, Paris. 1951. Price, 9,000 francs.

The complete work, of which the present volume is the first installment, will be a comprehensive account of the diseases of plants in the warmer regions of the earth, roughly that part in which the mean monthly temperature does not fall below 5° C.

As planned, there will be three parts. Part I, dealing with general considerations, discusses the nature of plant diseases, the classification of abnormalities, the general nature of parasitism and resistance and the principles of control. Part II treats of the fungi as causes of plant disease. After a general discussion of the nature and classification of fungi, the various groups and the diseases associated with them are considered in the taxonomic sequence adopted. In the present volume, this includes the Myxomycetes, Archimycetes, Siphonomycetes (Phycomycetes in the restricted sense) and Basidiomycetes. Diseases caused by Ascomycetes, imperfect fungi, algae, lichens, mosses and flowering plants and bacterial and virus diseases are to be included in a later volume or volumes, which will also embrace Part III, a general treatment of diseases and their control, arranged by crops, together with indexes.

An enormous amount of information on tropical diseases is included in the volume at hand and in view of the increasing importance of tropical agriculture, these volumes will undoubtedly be of great service wherever large-scale control of the diseases of economic plants in the tropics is being attempted.

Authors are cited freely, but there will be no bibliography. This is probably not too serious, since the names cited will be familiar to specialists and their writings would be largely unavailable to working plant pathologists in many parts of the world where the book will be used.—G. W. M.

MANUSCRIPT

Publication in MYCOLOGIA is ordinarily restricted to members of the Mycological Society of America. Exceptions to this regulation require a favorable vote by a majority of the Editorial Board. When a paper has two or more authors, the person submitting the paper is expected to be a member.

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All illustrations should be numbered consecutively throughout a paper, using arabic numbers and small letters for subdivisions, e.g., Fig. 1, a etc. This does not mean that all figures grouped for convenience on a single page need have a single number. Figures should be prepared so that, when reduced, the width will not exceed 4 inches, and should be short enough to permit the insertion of the legend beneath the figures. Each article will be restricted to twenty-five pages, including illustrations, except when authors submit only one paper in two or three years of membership, in which case the restriction will be forty and fifty pages respectively. Ruled tabular matter is counted double. Should an author wish to publish additional pages in one article he may do so by paying for the excess pages at current rates.

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To comply with the International Rules, it is recommended that contributors furnish *brief* Latin diagnoses of all new species and genera when their manuscript is submitted for publication.

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